Vol. XXXVI December, 1952 - January, 1953 Nos. 7-8

THE PHILIPPINE AGRICULTURIST

UNIVERSITY OF THE PHILIPPINES PUBLICATIONS SERIES A

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Published by

THE COLLEGE OF AGRICULTURE
AND CENTRAL EXPERIMENT STATION
UNIVERSITY OF THE PHILIPPINES

COMMONWEALTH MYCOLOGICAL INSTITUTE
24 MAY 1954

The Philippine Agriculturist

(University of the Philippines Publications Series A)

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THE PHILIPPINE AGRICULTURIST is published monthly, except in April and May, beginning with Volume 35, by the College of Agriculture and Central Experiment Station, University of the Philippines, Los Baños, Laguna. The subscription price is ₱6.00 a year in the Philippines and $4.00 (U.S. currency) elsewhere; the price of single copies, ₱0.75 in the Philippines and $0.50 (U.S. currency) elsewhere.

Business correspondence should be addressed to the Business Manager, THE PHILIPPINE AGRICULTURIST, College, Laguna, Philippines. All remittances should be made payable to THE PHILIPPINE AGRICULTURIST.

Communications for the editor should be addressed to the Editor, THE PHILIPPINE AGRICULTURIST, College, Laguna, Philippines.

Publications sent in exchange for THE PHILIPPINE AGRICULTURIST should be addressed: Library, College of Agriculture and Central Experiment Station, College, Laguna, Philippines.

Entered at the Post Office at College, Laguna, Philippines, as second-class mail matter.

LEAF SPOT OF MAIZE CAUSED BY HELMINTHOSPORIUM MAYDIS [1]

FAUSTINO T. ORILLO

WITH THREE PLATES AND SIX TEXT FIGURES

Leaf spot of maize, caused by *Helminthosporium maydis*, is a destructive and widely distributed disease. It occurs in China, Japan, India, the Philippines, Germany, northern Caucasus, the Gold Coast, Cuba, Canada, the United States, and perhaps wherever maize is grown. The damage caused by the disease varies in different places, perhaps on account of the differences in climate. The disease has been reported to infect as much as 90 per cent of the maize crop in some countries, and in others it has been known to occur in epiphytotic proportions.

The pathogen attacks maize plants at all stages of development, but it is particularly destructive on the seedlings which, when severely infected, usually assume a blighted appearance and eventually perish. On mature plants, the disease typically manifests itself on the leaves in the form of spindle-like or elliptical, light-brown to brown lesions which, although limited in size, are oftentimes so numerous as to cause a tremendous reduction in the photosynthetic activity of the leaves. Infection also occurs on the leaf sheaths and outer husks of ears, the symptoms on these plant parts being generally similar to those produced on the leaf blades. Although in the field the fungus normally does not affect the roots, stems, and midribs of mature leaves, it was able to cause infections of these various parts under very favorable conditions of artificial inoculation in the greenhouse.

The fungus grew and sporulated well on various agar media, such as potato-dextrose, oatmeal, malt extract, and corn meal agars, and on various sterilized plant tissues, such as potato cylinders, maize and teosinte stems and leaves, and string beans. It also grew well but sporulated sparingly in Richard's and Czapek's sodium nitrate-sucrose solutions.

Conidiophores and conidia of the fungus vary somewhat in shape, size, and septation, depending upon the medium or substrate on which they are produced and upon the environmental conditions under which they are developed. On diseased maize leaves incubated in a damp chamber, however, typical conidiophores and conidia are produced. The conidiophores emerge singly or in tufts of two to four from the stomata, are dark olivaceous to light brown in color and slightly lighter-colored towards the apex, are generally geniculate, and range in size from 102.5–376.5μ × 4.7–8.5μ with 3 to 18 septa. The conidia are typically fusoid to fusiform in shape, fuliginous to moderately olivaceous in color, 43.8–133.5μ × 9.8–16.5μ in size with 5 to 12 septa, strongly curved and moderately tapering toward the ends, with rounded basal and apical contours; the hilum is more or less internal and not protruding. Typical germination is by the production of two polar germ tubes.

To some extent, the fungus is capable of destroying cellulose in the laboratory. It grew under a wide range of temperature (10 to 40°C.), the

[1] Contribution from the Cryptogamic Laboratories, Harvard University, and the College of Agriculture and Central Experiment Station, University of the Philippines, No. 1687. Prepared from portion of the thesis written under the direction of Professor William H. Weston, Jr. and presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Harvard University, 1950.

[THE PHILIPPINE AGRICULTURIST, Vol. 36, No. 6, November, 1952, appeared in January, 1954.]
THE PHILIPPINE AGRICULTURIST, VOL. 36, Nos. 7-8, DECEMBER, 1952—JANUARY, 1953.

optimum range being 24 to 30°C. The thermal death point of conidia was 52°C. The fungus grew also under a wide range of pH (2.2 to 11.2), but it grew best at pH 5.8 to 6.4.

Cross-inoculations of several graminaceous species showed that *Euchlaena mexicana* and *Rottboellia exaltata* were markedly susceptible and, hence, can serve as potential reservoirs of infection for maize.

Penetration of the host tissues by the germ tubes occurred through stomata, through injuries, or directly through the uninjured epidermis. The germ tubes produced appressoria at the site of penetration. Tissue invasion was effected by the proliferation of the intracellular hyphae, causing the disorganization of the contents and the shrinking and discoloration of the walls of the mesophyll cells.

The mycelium within host tissues, mixed with dry or with watered soil in the greenhouse, remained viable for 20 and 10 months, respectively. Conidia stored inside glass jars in the laboratory lost their viability after 26 months; those mixed with dry soil in the greenhouse lost their viability after 14 months. Experiments showed that conidia of the fungus can be disseminated by wind, rain, insects, and other agents, wind being perhaps the most efficient spore-dispering agent.

Of four protectant fungicides tested in the laboratory, Bordeaux mixture and Semesan were promising, and when sprayed on maize plants in the greenhouse, they protected the plants adequately against infection by the fungus. Aside from spraying, preferably with Bordeaux mixture, the disease can be reduced, if not eliminated, by the removal of infected remains of maize from the field, by destruction of probable wild hosts, and by planting of resistant varieties of maize.

Leaf spot of maize (*Zea mays* L.), caused by the fungus *Helminthosporium maydis* Nisikado and Miyake, is widely distributed and has become established in many parts of the globe differing in climate and other factors of the environment. In the Philippines leaf spot usually becomes alarmingly serious on some native and introduced maize varieties when conditions are favorable for disease development.

In view of the threatening potentialities and the widespread occurrence of the disease, the writer's investigation was undertaken in an effort to contribute to the present rather limited knowledge of the disease and also to provide a basis for more effective methods of control.

THE DISEASE

Economic importance, history, and geographic distribution

Leaf spot of maize was first reported by Drechsler in 1925 on materials collected from Florida and from the Philippines. By artificial inoculation experiments, Drechsler demonstrated the vigorous parasitism of the fungus on maize and described the causal organism in its conidial and ascigerous stages. The conidial phase he merely referred to, without specific designation, as *Helminthosporium*, but on the basis of the ascigerous phase, he named the fungus *Ophiobolus heterostrophus*. Later, on revising *Ophiobolus*, he established a new genus, *Cochliobolus*, for the fungus and changed its name to *Cochliobolus heterostrophus* (Drechsler, 1927, 1934).

In 1926, Nisikado and Miyake reported the widespread occurrence of the disease in Japan and for the first time named the imperfect form of the causal fungus *Helminthosporium maydis*. In East China, during the wet season of 1925, a leaf spot of maize attributed to a species of *Helminthosporium*, possibly referrable to *H. maydis*, was reported by Porter (1926) to have caused 7 per cent loss in the crop. Also in China, Yu (1933) found that during a two-year survey of the Nanking district, over 90 per cent of the corn crop was infected in varying degrees of severity by the leafspot fungus. Takasugi (1934), in his notes on the fungi of Manchuria, listed *H. maydis* as infecting maize in that region. Likewise, the fungus was listed by Lee Ling (1948) as one of the parasitic fungi on maize in Szechwan, China.

The fungus was found by Bunting (1926, 1928) to infect maize in the Gold Coast. Lobik (1933), reporting on the results of a survey started in 1929, states that *H. maydis* was one of the numerous fungi, parasitic and saprophytic, on maize in the northern Caucasus. In Germany, Boning (1938) reported a severe outbreak of brown spotting, which caused premature death of leaves, on various maize varieties growing in two localities in southern Bavaria. The causal fungus was identified as *H. maydis*.

Ocfemia (1931) and Silayan (1938) reported the occurrence of *H. maydis* on maize in the Philippines. As far as the writer is aware, these reports constitute the first two published records of this fungus in the Philippines since Drechsler's (1925 a & b) original account of the pathogen occurring on diseased maize specimens collected from the Philippines. The writer, however, has observed the leaf spot on practically all native and introduced varieties of maize grown in the experiment station and vicinity of the College of Agriculture, Los Baños, Laguna. In a preliminary study from 1943 to 1944, he observed that the disease was serious on the native maize variety Lagkitan.

While the writer was in Soledad, Cuba, in the summer of 1948, he found that the disease was present on sweet corn variety U.S.D.A. 34 and, to some extent, on the native Cuban corn (maize Criollo) growing in the Atkins Garden. The disease was further observed to be prevalent, with such severity as to interfere seriously with the normal development of the young plants, on practically all of the Mexican strains of corn which at that time were being tested in the Garden.

In the United States, the disease was obviously present in Florida before or in 1923, since Drechsler (1925 a & b) described the causal organism partly on the basis of materials collected from that state on September 22, 1923. In 1930, Eddins noted that leaf spot was one of the destructive maize diseases occurring in Florida. Young (1943) reported the occurrence, in the late summer of 1942, of a relatively severe epidemic of leaf spot of corn caused by *H. maydis* in the southeastern states. In 1946, Arndt and Stevenson observed

that leaf spots of corn caused by both *H. maydis* and *H. turcicum* were prevalent throughout South Carolina. Wernham reported the disease for the first time in Pennsylvania in 1946. He states that in 1945 *H. maydis* caused the most serious leaf blight of corn in southeastern Pennsylvania and the southern areas of New Jersey and that at Avondale, Chester County, the disease assumed epiphytotic proportions.

Richardson (1942) found that *H. maydis* was among the several fungi which constituted a root-rotting fungous complex in the corn fields of Ontario, Canada. He demonstrated conclusively by means of soil inoculation with pure cultures of the fungus the ability of *H. maydis* to cause corn root rot.

H. maydis has also been reported to cause diseases of plants other than maize. In India, Mitra (1930, 1931 b) reported the occurrence of a strain of *H. maydis* parasitic on ginger (*Zingiber officinale* Rosc.). During the rainy season, the disease was observed to appear on this host in the form of small oval spots which measured 4 to 5 by 2 to 3 mm. and which were scattered over both surfaces of the leaves. After the rains, the spots were observed to develop rapidly and show a brownish center and a diffuse yellowish margin. The mature ones consisted of a central dead, straw-colored area surrounded by a brown ring with a yellowish outer zone. Under experimental conditions, this strain of *H. maydis,* which freely parasitized ginger, was found to be unable to cause root rot on wheat and barley, in contrast to *H. sativum, H. bicolor, H. halodes* var. *tritici, H. sacchari,* and *H. oryzae* which all gave positive results. The author, however, did not test its pathogenicity on maize. Mitra (1931a) also reported the occurrence of *H. maydis* on sorghum in the Punjab. Padwick (1938) conducted inoculation tests with *H. maydis* and found that pea cotyledons were highly susceptible to the fungus. Seymour (1929) lists this fungus as naturally occurring on teosinte (*Euchlaena mexicana* Schrad.) in North America.

Symptoms

In its early stages, the disease manifests itself on the young or mature leaves of the host plant as water-soaked, more or less circular spots ranging in diameter from 1.0 to 2.5 mm. The center of a spot is occupied by a minute speck slightly lighter in color than the surrounding tissues. As the disease progresses the spots gradually enlarge generally along the longitudinal axis of the leaf, lateral extension being usually, but not always, checked by the veins. As a result of the more rapid longitudinal enlargement of the infected areas, elongate lesions are formed, usually with the lateral edges parallel and coinciding with the veins bordering a single intervascular region. Old lesions may be roughly rectangular, spindle-like, or elliptical in shape and vary in size anywhere from 5 to 15 mm. in length and 1.5 to 3.0 mm. in width (Pl. I, fig. 1). They are, on the whole, from light brown to brown in color with two or three

rather distinct, concentric, reddish-brown zonations and a darker reddish-brown margin which sets off the individual lesions from the surrounding healthy tissues. Frequently, neighboring lesions may coalesce end to end and side by side, resulting in the formation of dead areas which occupy two adjacent intervascular spaces. The formation of such necrotic patches covering more than one intervascular region seldom, if ever, occurs through the lateral extension of single lesions. As pointed out previously, this frequent limitation in lateral enlargement of any one lesion is due to the veins which often serve as barriers against further growth of the pathogen within the infected tissue.

The relatively small size of the lesions is more than compensated for by their number. It is not unusual to observe a heavily infected mature leaf almost completely covered with lesions, ranging in number from 100 to 400 depending upon the area of the leaf. For instance, a count made on a leaf, which had been severely infected under natural conditions and measured 58 cm. in length and 3 cm. in width at the broadest portion of the blade, showed the presence of 273 spots of different sizes. The total necrotic area composed of the numerous lesions on any one leaf is thus seen to constitute a considerable portion of the leaf. This vast aggregate of dead and nonfunctional tissues, therefore, causes a tremendous reduction in the photosynthetic activity of the leaf. The situation becomes worse when the plant is infected also with the leafblight fungus, *Helminthosporium turcicum,* which, in contrast to *Helminthosporium maydis,* attacks the same host usually in its maturity. Thus a maize plant, when severely infected even with *H. maydis* alone during its early development, either perishes eventually or, if it ever survives, becomes stunted and presents a generally unthrifty appearance. The infected plant which has survived is usually able to produce a normal, fully grained ear, but as a rule, the ear is much smaller than that produced by a healthy plant.

In nature, when conditions are favorable for sporulation of the fungus, the old lesions on the host leaves become grayish and lose the characteristic brown or reddish-brown coloration. Examination with a hand lens reveals that these lesions are covered with myriads of fungous fructifications—conidiophores and conidia—which, moreover, are present not only within the limits of the lesions but also on the surrounding apparently healthy tissues. The grayish tinge presented by the vast number of fruiting bodies may later turn velvety if the favorable conditions continue to prevail for some length of time.

Observations of fully grown infected plants in the field have shown that lesions are generally more numerous on the lower and older leaves than on the upper and younger leaves. No attempt has been made by the writer to correlate the number of lesions on the leaves with the position of the latter on the stem of the plant or with the height of the leaves from the ground. In his opinion,

however, a probable explanation for the presence of more lesions on the older and lower leaves could be sought in the role of rain splash in spore dissemination. It may be that during heavy rains the spores which are washed down and fall to the ground are splashed back to the leaves by the rain water. Since the splash from the ground seldom, if ever, reaches the upper leaves, the spores that it carries are probably deposited in greater number on the lower leaves which are more exposed to the splashing action. The abundant spores then deposited in this manner would account for the more numerous infections of the lower leaves in contrast to the relatively fewer infections of the upper leaves.

The fungus can attack maize plants at all stages of development, from the seedling to the fruiting stage. It is, however, particularly destructive to seedlings from the time of emergence until they are a foot or so high (Pl. I, fig. 2). When severely infected during this stage, the seedlings invariably succumb to the disease, generally assuming a blighted appearance prior to death. The characteristic foliar spotting is, in most instances, not distinct on seedlings, for the young succulent leaves tend to shrivel and wither before the lesions become fully differentiated. Only on older and less tender leaves are the lesions more defined.

Infection by the fungus is not confined to the leaf blades alone. Leaf sheaths are also attacked and during the fruiting stage of the host plant, the green outer husks of the ears are similiarly attacked. The symptoms of the disease on the leaf sheaths and husks of ears are more or less identical with those appearing on the leaf blades.

In the field, the writer has never observed the fungus to cause infection on the roots, stems, and midribs of mature leaves. In the greenhouse, however, infections of young stems and midribs of mature leaves were obtained after these plant parts had been wounded and the plants subjected to extremely humid conditions after inoculation. Also in the greenhouse, root infections were obtained when plants were grown in soil previously inoculated with pure cultures of the fungus. Moreover, planting seeds in inoculated soil resulted in a reduction in the stand because of pre-emergence killing and in the stunting of the plants because of the parasitic invasion and destruction of their roots by the pathogen.

THE CAUSAL ORGANISM

Isolation and identification of the causal fungus

The fungus was easily isolated by planting bits of infected tissue on agar. Sections of either fresh or dried infected leaves measuring approximately 3 mm. sq. were disinfected by immersion in a 1:1000 mercuric bichloride solution for about 5 minutes. Then they were washed in three changes of sterile distilled water and plated out on sterile potato-dextrose or corn meal agar. Four of such sections were placed equidistantly in one Petri plate. After an incubation period of 72 hours at room temperature, hyphae

grew from the leaf sections onto the agar medium. From these young hyphal growths, transfers were subsequently made on agar slants or plates. Cultures obtained thus were invariably free from bacterial or fungous contaminants.

Isolation of the fungus was also readily made by disinfecting larger sections of infected leaves, washing them thoroughly with sterile water, and incubating them in Petri dishes lined at the bottom with moist filter paper. From these leaf sections, abundant growths of conidiophores and conidia usually ensued within a week. Since the sections had been surface-sterilized, the fungus growing from them was practically free from contaminants. Conidia could then be picked up with a micro-needle under a dissecting microscope and transferred to a suitable agar medium. The writer experienced no difficulty in making single-spore isolations by this method.

The fungus was also isolated successfully by using conidia produced on lesions in the field. The conidia were scraped off from lesions of naturally infected leaves and suspended in sterile water. The resulting spore suspension was then spread thinly over a thin layer of water agar in a Petri dish, and individual conidia were again picked up with a micro-needle and transferred to the desired medium. In some cases, the conidia were allowed to germinate first on the water agar and transfers made from the hyphae which subsequently developed from the viable conidia. Since the leaf sections from which the conidia were obtained were not previously disinfected, bacterial contaminants occasionally occurred in the transfers. Pure cultures, however, were finally secured by transferring the conidia or hyphae on agar medium acidified with lactic acid, which prevented the bacteria from growing and interfering with the fungus.

In the present work, four isolates of the pathogen were studied: (1) isolate from the College of Agriculture, Los Baños, Laguna, (2) isolate from Pennsylvania State College, (3) isolate from the U.S. Bureau of Plant Industry, Soils, and Agricultural Engineering, Beltsville, Maryland, and (4) isolate from Cuba. The isolation of (1) and (3) was done by the writer from diseased specimens furnished by Mr. W. L. Fernandez and Dr. J. A. Stevenson, respectively. Similarly, isolation of (4) was made by the writer from infected sweet corn variety U.S.D.A. 34 which he collected at the Atkins Garden, Soledad, Cuba, in the summer of 1948. Isolate (2) was furnished as pure culture by Dr. C. C. Wernham. Throughout this paper, isolates (1), (2), (3), and (4) will be referred to as Philippine, Pennsylvania, Beltsville, and Cuban isolates, respectively.

The fungus isolated by the writer from the diseased maize specimens collected in the Philippines, Beltsville, and Cuba is identical with the Pennsylvania isolate, which had been identified by Dr. C. C. Wernham as *H. maydis*. Moreover, these isolates agree closely with

the earlier descriptions, morphological and pathological, of *H. maydis* given in the literature (Drechsler, 1925a & b; Nisikado and Miyake, 1926; Yu, 1933). The writer, therefore, concludes that the four isolates of the fungus under study belong to the same species—*Helminthosporium maydis* Nisikado and Miyake.

Morphology

Conidiophores. In nature and on diseased materials incubated in damp chambers in the laboratory, conidiophores are produced aerially from the intracellular hyphae and emerge either singly or in tufts of two to four from the stomata on either the upper or the lower epidermis of the leaves (Pl. I, figs. 3-6; Pl. II, figs. 1 and 2). Conidiophore emergence seems to be restricted to the stomata. It is a rare occurrence indeed when conidiophores are observed to emerge directly through the epidermis. Whether or not this limitation to stomata is an inherent characteristic of the fungus is not known, but the writer is inclined to agree with Drechsler (1925a & b) that such limitation can be regarded as due to the mechanical firmness of the maize epidermis which makes it quite difficult for the conidiophores to break through.

Young septate conidiophores which have just emerged from the stomata are not sharply differentiated from the subhyaline or fuliginous hyphae from which they arise, except for the constriction at the point where resistance was encountered in their passage through the stomatal pore and for the distinctly swollen basal cell immediately above the point of emergence. The walls of young conidiophores are thin like those of the parent hyphae, and the contents are dense and finely granular and very rarely vacuolate. As the conidiophores mature, their walls thicken and the contents become vacuolate and less dense. Moreover, the walls become darker than those of the parent hyphae. Mature conidiophores are dark olivaceous but are slightly lighter towards the apex. They gradually turn light brown to brown as they become older. The conidiophores become geniculate at the points where conidia have been budded off (Pl. I, fig. 5). The geniculations, bearing the scars which mark the former points of attachment of conidia, are not always pronounced. They occur at relatively wide intervals of 12 to 50 μ, the first one being produced usually at a distance of 45 to 60 μ from the base of the conidiophore (Pl. III, figs. 1 and 2).

Conidiophores produced in agar cultures differ from those produced on host tissues not only in their greater length and greater number of septa, but also in the absence of the conspicuously swollen basal cell. These conidiophores originate as branches or prolongations of prostrate hyphal filaments growing on the surface of the agar medium (Pl. II, figs. 3 and 4) and can be distinguished from the latter by their thicker walls and darker coloration. Branching of the conidiophores sometimes occurs and, as on the main sporophoric filament, conidia are also developed on the lateral branches

(Pl. II, fig. 6). In all other respects—geniculate appearance, width, coloration, and characteristics of cell contents—the conidiophores developed in pure cultures on agar media resemble those developed in nature.

In pure cultures, conidiophores have never been observed to arise from hyphal filaments which are completely submerged in the agar medium. This observation suggests that aeration is essential to conidiophore and, consequently, to conidial formation in culture. That such is the case seems to be borne out by the fact that the fungus does not sporulate in liquid cultures when the mycelium is totally submerged and by the fact that in slide cultures, where the inoculum is embedded within the thin layer of agar and deprived of adequate aeration by a cover glass on top, sporulation occurs only after the hyphae have grown out along the edges of the layer of agar.

The size and septation of conidiophores vary under different growth conditions, and even under one set of conditions some variation also exists (table 1). The length of conidiophores varies markedly, the conidiophores produced in agar cultures being, on the average, almost twice as long as those produced under natural conditions and those developed on infected materials incubated in damp chambers being more or less intermediate between the two. There is no significant difference in width, the conidiophores from the three lots being approximately equal in this dimension. The conidiophores produced in agar culture are, on the average, more septate than those in the other two lots, indicating that there is a direct relation between conidiophore length and number of septa.

Measurements of conidiophores of *H. maydis* have been reported by Drechsler (1925a & b)as 120-170 μ (sometimes 1 mm.) $\times$ 4.5-7.0 μ with septa occurring at intervals of 15 to 60 μ; by Nisikado and Miyake (1926) as 162.4-487.2 μ $\times$ 5.1-8.9 μ (average, 294.4 μ $\times$ 7.2 μ) with 4-17 septa (average, 10.1) ; and by Yu (1933) as 92.2-237.4 μ $\times$ 5.9-8.2 μ with 3-18 septa (usually 5-11). These measurements were made by the investigators using conidiophores from naturally infected materials incubated in damp chambers in the laboratory. As seen in table 1, the writer's average figures on the length, width, and number of septa of conidiophores produced on naturally infected leaves incubated in a damp chamber compare favorably with those of the other workers.

Conidia. The conidia of the fungus produced on diseased materials under natural conditions, on infected materials placed in damp chambers in the laboratory, or in pure cultures growing on various agar media kept under ordinary laboratory conditions are typically fusoid to fusiform in shape, the widest point being frequently located at the center of the conidia. They are strongly curved and taper moderately toward the ends so that the base and the apex present rounded contours (Pl. II, figs. 6 and 7). The hilum or the scar which marks the point of attachment of the conidium to the conidiophore is rather broad and flat and does not protrude from the rounded contour

of the basal end of the conidium. Young conidia have thin peripheral walls, especially in the basal and apical regions, and their contents are finely granular and dense (Pl. II, fig. 3). With age the walls become thicker and the contents less dense and vacuolate (Pl. II, fig. 4). Mature conidia, regardless of whether they are produced under natural conditions or in the laboratory, are generally fuliginous to moderately olivaceous in color. Very old conidia usually assume a light brown to brown coloration.

Even under strictly identical conditions, the conidia vary in size and septation. There is a slight increase in the length and width of conidia depending on the conditions under which they are produced, those produced in the laboratory tending to be longer and wider than those developed under natural conditions (table 2). The same trend seems to occur as regards the number of septa, although the variation is much less apparent. The ranges and averages of size and septation as reported here are in close agreement with those reported for this fungus by Drechsler (1925a & b), 30-115 $\mu \times$ 10-17 μ with a limit of 12 septa; by Nisikado and Miyake (1926), 40-130 $\mu \times$ 10.2-21.6 μ (average, 87.4 $\mu \times$ 14.7 μ) with 2-15 septa (average, 8.1) ; and by Yu (1933), 46.4-138 $\mu \times$ 11.9-19.4 μ with 2-12 septa (usually 82.2-105.8 $\mu \times$ 13.8-14.7 μ with 6.8 septa).

Germination of conidia. Germination of conidia is typically bipolar, that is, by the production of a single germ tube from each end of a conidium, the tube arising from the apical end usually protruding in advance of that arising from the basal end (Pl. III, fig. 7). Germination by the production of a single germ tube from either the basal or the apical cell, usually from the latter, is also frequently observed (Pl. II, fig. 5; Pl. III, fig. 4). Atypical methods of germination, such as production of two germ tubes from either or both ends, two tubes from one end and one tube from the other, one germ tube from either or both ends plus one or two lateral tubes from any of the intermediate segments, or two tubes from either end plus one or two lateral tubes from an intermediate segment (Pl. III, figs. 3-9), have likewise been observed, but less frequently than the first two methods.

It should be added that conidia, being supplied with sufficient storage material, germinate vigorously in distilled water in the laboratory. The addition of nutrients or stimulants in the spore suspension, therefore, has been dispensed with in all experiments involving germination of conidia.

Results of observation on the methods of germination of 500 four-week-old conidia mounted in sterile distilled water in van Tieghem cells and incubated for five hours at 27°C. are given in table 3. As seen from these results, conidia of *H. maydis* seem to possess no definite polarity in regard to germination. This contention is substantiated by the fact that conidia germinate atypically, although not too frequently, regardless of whether they are injured or not. In-

jury is apparently not necessary in inciting the production of lateral germ tubes from any of the intermediate segments of the conidia.

Atypical germination of conidia is a characteristic found not only in *H. maydis* but in other species of the genus *Helminthosporium* as well. Drechsler (1923) observed atypical conidial germination in 18 species of *Helminthosporium* which included *H. gramineum, H. teres, H. avenae, H. turcicum, H. oryzae,* and others.

Conidia which are about to germinate can be readily distinguished from the dormant ones by the conspicuous thinning of the portion of the cell wall from which the germ tubes are to protrude. The thinning of the wall is accompanied by a reduction in the intensity of the normally dark coloration of the wall. A dissolving action seems to take place at the points of germ tube extrusion. The germ tubes produced by either typical or atypical methods of germination are 2.5 to 4.5 microns in diameter, hyaline, thin-walled, and less frequently septate after attaining a length of 100 or more microns. The cell contents are very finely granular and dense, with or without minute vacuoles. The commencement of branching of the germ tubes is not fixed, the first branch being produced either immediately or much later after the tubes have been extruded from the peripheral wall of a conidial segment. Branching and rebranching take place until the reserve food in the germinated conidia is exhausted, at which stage the activity of the profusely branched germ tubes, ceases if nutrients from the surrounding medium are not available. On host tissues, appressoria-like structures are produced at the tips of the germ tubes.

Physiology

Growth on various solid and liquid media and plant tissues. Helminthosporium maydis grew well and sporulated profusely on potato-dextrose and corn meal agars. On these media, however, only the conidial stage of the fungus was observed. To determine whether the fungus could produce the ascigerous stage on other substrates, it was grown on different solid and liquid media as well as on various sterilized plant tissues. The four isolates of *H. maydis* were grown on malt extract, oatmeal, prune, lima bean, rice meal, rice leaf decoction, and maize leaf decoction agars; on sterilized potato cylinders, maize, rice, and teosinte stems; and in Richard's and Czapek's sodium nitrate-sucrose solutions. On all of the agar media and sterilized plant tissues, the fungus produced abundant mycelium and conidia, but no perithecia. In the two solutions it also grew well, but sporulated sparingly and did not produce perithecia. A synthetic medium which was found by Westergaard and Mitchell (1947) to favor sexual reproduction of *Neurospora* was also tried. Although *H. maydis* grew satisfactorily on this medium, it again failed to produce the ascigerous stage. Other attempts to induce the production of the perfect stage of the fungus by subjecting pure

cultures, as well as infected maize leaves, to different conditions of temperature, pH, light, humidity, and desiccation gave negative results. Similarly, negative results were obtained when the four isolates were grown together in different combinations on the same culture plate.

Ability of the fungus to destroy cellulose. When sections of infected maize leaves were incubated inside Petri dishes lined at the bottom with moist filter paper, the writer noted that the hyphae of the fungus frequently radiated from the cut edges of the leaf sections onto the filter paper and thereupon grew and sporulated, although rather sparsely. When the leaf sections were removed from the Petri dish and the filter paper was kept constantly moist, the strands of hyphae left on the paper continued to grow slowly, occasionally sending out conidiophores upon which conidia eventually developed. Since the only possible source of carbon for growth of the fungus was the filter paper and since filter paper is almost wholly made up of pure cellulose, it was thought that the fungus might be capable of digesting cellulose, at least to some extent, and converting it into forms more utilizable for growth.

Cases are known of pathogenic species being able to destroy cellulose. For instance, Satoh (1931) found that *Piricularia oryzae, Ophiobolus miyabeanus, Helminthosporium* sp., two strains of *Fusarium* sp., *Gibberella saubinetii,* and *Rhizoctonia papayae* were able to decompose cellulose. With the exception of *Helminthosporium* sp. and the two strains of *Fusarium* sp., all the foregoing fungi are confirmed plant pathogens. Seto (1936) found that cellulose decomposition was effected by *Corticium centrifugum, Fomes applanatus, Gibberella fujikuroi, Pestalozzia diospyri, Polystictus pergamenus,* and *Polystictus sanguineus.* He further observed that among the fungi tested, *Gloeosporium olivarum, Pestalozzia theae,* and *Sclerotium oryzae* were most active as cellulose destroyers.

In view of the observation that *Helminthosporium maydis* mycelium grew on the filter paper in the plates used in isolating this pathogen, experiments were performed to test the cellulolytic activity of *H. maydis.* The procedure employed by the writer was basically similar to that used by White *et al.* (1948). Two nutrient solutions were used, namely, the solution designated by White *et al.* as "Formula A" and Czapek's sodium nitrate solution. The composition of the two solutions is as follows:

FORMULA A		CZAPEK'S SODIUM NITRATE SOLUTION	
K_2HPO_4	1.3940 gm.	$NaNo_3$	2.00 gm.
$MgSO_4.7H_2O$	0.7395 "	K_2HPO_4	1.00 "
NH_4NO_3	1.0006 "	KCl	0.50 "
$CaCO_3$	0.0050 "	$MgSO_4.7H_2O$	0.50 "
NaCl	0.0050 "	$FeSO_4$	0.01 "
$Fe_2(SO_4)_3.7H_2O$.	0.0010 "	Distilled water	1 liter
$MnSO_4$	0.0010 "		
$ZnSO_4.7H_2O$	0.0010 "		
Distilled water ..	1 liter		

To each of these solutions, 1.5 per cent agar was added.

Modified French square bottles stoppered with cotton plugs were used. Twenty ml. of medium was placed in each bottle and autoclaved for 20 minutes at 15 pounds pressure. Dry strips of white muslin cloth, cut to rectangular pieces measuring 8.5 cm. × 2 cm., were thoroughly washed to remove the sizing, placed in a separate container, and similarly autoclaved. When the bottles had cooled and the medium congealed, a strip of the fabric was transferred aseptically to each bottle by means of a pair of forceps. The bottles thus prepared were inoculated by pipetting 1 ml. of spore suspension into them and distributing the suspension uniformly over the surface of the strip of cloth.

The conidia used as inoculum in these experiments were obtained from three-week-old potato-dextrose agar slant cultures of three isolates of *H. maydis,* namely, the Philippine, Pennsylvania, and Beltsville isolates. Controls were provided for all experiments. One ml. sterile distilled water was pipetted into each control bottle. The inoculated as well as the control bottles were kept inside a wooden cabinet in the laboratory, and the fungus was allowed to grow at room temperature. At weekly intervals, the extent of growth of the fungus was observed and on the fourth week, the degree of deterioration of the fabric was determined. The writer did not use any of the standard machines, such as the Scott tester, for determining the decline in tensile strength of the fabric, but roughly estimated the deteriorating action of the fungus by measuring with a spring balance the amount of force necessary to break the fabric. The culture bottle was flooded inside with a 1:1000 mercuric bichloride solution for 10 minutes to kill off the fungus growing on the strip of cloth. Then the strip was taken out, rinsed in tap water, and air-dried. One end of the strip was tied securely to a spring balance; the other end was likewise tied with a piece of twine and pulled with the hand. In this way, the approximate breaking point of the strip was registered on the balance. Four strips for each of the three isolates of the fungus were tested for their resistance to breaking. The results of the experiments are presented in table 4.

The strips of muslin cloth upon which the fungus had grown for four weeks showed a considerable decline in tensile strength, the controls retaining a strength more than eight times that of the inoculated strips (table 4). The results are undoubtedly an indication of the ability of the fungus to digest cellulose. Hyphae were observed to be intimately interwoven with the fibers comprising the strips of cloth. Beyond this observation, the writer did not study in any great detail the precise mechanism by which *H. maydis* caused the degradation of the fabric tested. No work was done to determine whether the hyphae penetrated into the lumina of the fibers or merely grew outside of but in close apposition to the fibers.

The three isolates of the fungus were further grown on the same kind of fabric but supplied with nutrients in Czapek's sodium nitrate

solution. Compared to the growth obtained on fabric with "Formula A" nutrients, the growth of the fungus on fabric with Czapek's nutrients was slightly poorer. But despite this difference in growth, the fungus still caused significant deterioration of the latter fabric, as evidenced by the considerable reduction in its tensile strength at the end of four weeks.

The three isolates were also grown in Czapek's sodium nitrate solution without any carbohydrate component and with filter paper as a source of carbon. For comparison, the fungus was grown on the same nutrient solution supplied with 20 gm. of dextrose per liter as source of carbon and on the same solution supplied with 10 gm. of cellobiose per liter. Fifty ml. of solution with the respective carbon source was put in a 125-ml. Erlenmeyer flask which was plugged with carbon and autoclaved for 20 minutes at 15 pounds pressure. It should be mentioned that the 9-cm. diameter filter papers used in the experiments were folded in such a way that when one was inserted into a flask containing the solution, the surface of the paper was not entirely submerged. Upon cooling, the autoclaved flasks were inoculated by pipetting into each of them 1 ml. of spore suspension of each fungous isolate which had been grown for three weeks on potato-dextrose agar slants. For each of the three isolates, three flasks of each solution were inoculated. The flasks were incubated for four weeks in the laboratory at room temperature, after which visual comparison of the fungus growths was made. The results are presented in table 5.

The three isolates scarcely grew in the complete absence of carbon, growth having apparently ceased after the reserve food in the inoculum had been exhausted (table 5). Growth was improved when filter paper was added to the nutrient solution, a positive indication that to some extent the fungus was able to utilize cellulose as carbon source. It is interesting to note that the Pennsylvania isolate utilized cellulose to better advantage than the Philippine and Beltsville isolates, as evidenced by its abundant growth on the solution with filter paper. The addition of cellobiose in the nutrient solution further enhanced the growth of the three isolates, the Pennsylvania isolate producing more abundant growth than the Philippine or the Beltsville isolate. Eventually the Pennsylvania isolate can convert the less digestible forms of carbohydrate like cellobiose into more assimilable forms more efficiently than the Philippine and Beltsville isolates. When dextrose was supplied as carbon source, all three isolates grew very abundantly with hardly any difference in the amount. With the Pennsylvania isolate no significant difference in amount of growth was noted when either dextrose or cellobiose was added to the solution. This observation indicates that this isolate utilized dextrose to the same degree as it did cellobiose. In contrast, the Philippine and Beltsville isolates manifestly preferred dextrose to cellobiose since their growth was markedly better on the solution supplied with dextrose than on the solution supplied with cellobiose.

A positive indication of the ability of the fungus to decompose cellulose was also obtained by testing the nutrient solution, to which filter paper had been added, for reducing sugar at the end of the four-week growth period. What solution remained in the flask was diluted to twice its volume with distilled water and passed through a Seitz filter to remove the mycelial fragments. A faint but nevertheless positive reaction was shown by the filtered solution when tested with either Fehling's or Benedict's reagent. The uninoculated solution, to which filter paper had also been added, gave negative reaction when similarly treated. This conclusively shows that the cellulose comprising the filter paper had undergone slight hydrolysis while the fungus was growing on it. Hydrolysis was most probably accomplished by the fungus by means of an enzyme or enzyme system.

Effect of temperature on mycelial growth and sporulation. Temperature is one of the most important factors, and frequently a limiting one, affecting the vital processes of fungi. It has been shown to affect markedly the fungus growth rate and sporulation and, to some extent, the morphological characters of both vegetative and reproductive structures. Among many plant pathogens, temperature likewise influences virulence. The thermal relation to virulence of numerous parasitic fungi has been studied by many investigators.

Studies on the effects of temperature upon species of the genus *Helminthosporium* have not been wanting. For instance, Ravn (1901) found that the maximum temperature for growth of *Helminthosporium gramineum* was 33-34°C.; the optimum, 25-30°C.; and the minimum, 3-5°C. Thermal relation studies on *H. oryzae* have been made by Nisikado and Miyake (1922) and by Ocfemia (1924a, 1924b). Stevens (1922) and Dosdall (1923) studied temperature effects on *H. sativum.*

In the present work, the writer studied the effect of varying temperatures on the growth and sporulation of *H. maydis* by growing the fungus in a series of constant temperature chambers (cold rooms and incubators) ranging from 5 to 40°C. The medium used was corn meal agar contained in horizontal glass tubes patterned after those employed by Ryan, Beadle, and Tatum (1943). As pointed out by these investigators, the tube method is particularly useful in studying the growth rates of rapidly growing organisms, such as *Neurospora,* for which extra-large plate cultures would be required. In the present work, the technique also proved suitable for measuring the growth rate of the fast-growing *H. maydis.* Another advantage of the method is that it greatly reduces the chances of the cultures' becoming contaminated—a misfortune all too frequently accompanying the examination of plate cultures when the covers are not taped to the inner plates and are likely to be lifted accidentally.

The growth tubes used by Ryan *et al.* were made of pyrex glass tubings with an internal bore of about 13 mm. The tubes were cut

into 40-cm. lengths and the terminal segments of 5 cm. were bent up at an angle of 45°; the openings were fire-polished and stoppered with cotton plugs. The tubes were held upright on a flat surface with spring clothes pins or metal clips. In the present experiments, the growth tubes were essentially similar to those already described, except that ordinary, not pyrex, glass tubings with an internal bore of about 11 mm. were used and pipe cleaners, instead of clothes pins or metal clips, supported the tubes. Keeping the tubes in an upright position was made possible by tying together the corresponding bent terminal segments of two tubes with a piece of the pipe cleaner.

Enough corn meal agar was added to each tube so that the horizontal portion was half-filled. The fungus was thus provided with a growth surface measuring approximately 30 cm. × 11 mm. The tubes were plugged with cotton and autoclaved for 15 minutes under 15 pounds pressure. One day after sterilization, the growth tubes were inoculated with *H. maydis*. The inoculum consisted of actively growing hyphal tips taken from the periphery of a one-week-old plate culture of the fungus. A bit of agar medium, small enough not to influence the initial growth of the fungus, was carried over with the hyphal tips from the parent culture. All the inoculated tubes were then kept at room temperature for 24 hours to allow the fungus to establish itself on the medium and resume its growth which had been interfered with by the transfer from the parent culture to the growth tubes. After this incubation period, when a uniform growth of mycelium extending to a few millimeters had been obtained, the advancing mycelial frontiers in each tube were marked off with a wax pencil. These marks served as starting points for the measurements of the subsequent growths of the fungus at a given temperature. The reference points having been made, two pairs of tubes were immediately placed in each constant temperature chamber having the desired temperature.

On the second day after the tubes had been transferred to their corresponding chambers, measurements of the linear growth of the fungus were made. Subsequent measurements were taken on the fourth, sixth, and eighth days. The last measurements had to be taken on the eighth day because the fungus growing under favorable temperature had almost covered the entire length of the horizontal portion of the tube. On the eighth day also, final observations on the relative abundance of aerial mycelium and conidia were made. The abundance or scarcity of conidia was determined by examining the tube under a dissecting microscope. No difficulty was encountered in examining the tubes for the presence of fruiting structures, for the conidiophores and conidia were of such a size that they could be seen clearly even under low magnification.

The results of the two series of experiments, conducted at one-month intervals and involving a total of 44 tubes for all the temperature conditions, showed that the minimum temperature for mycelial

growth of *H. maydis* is in the vicinity of 5°C. (table 6 and fig. 1). Judged by the linear growth made by the fungus at 10°C., the minimum temperature is probably somewhere above 5°C. but below 10°C. Since no cultures were kept between these two low temperatures, the precise minimum limit could not be ascertained. Although mycelial growth was obtained at 10°C., the fungus did not sporulate at this temperature. Sporulation was observed at 15°C., but there was an extreme paucity of conidial formation.

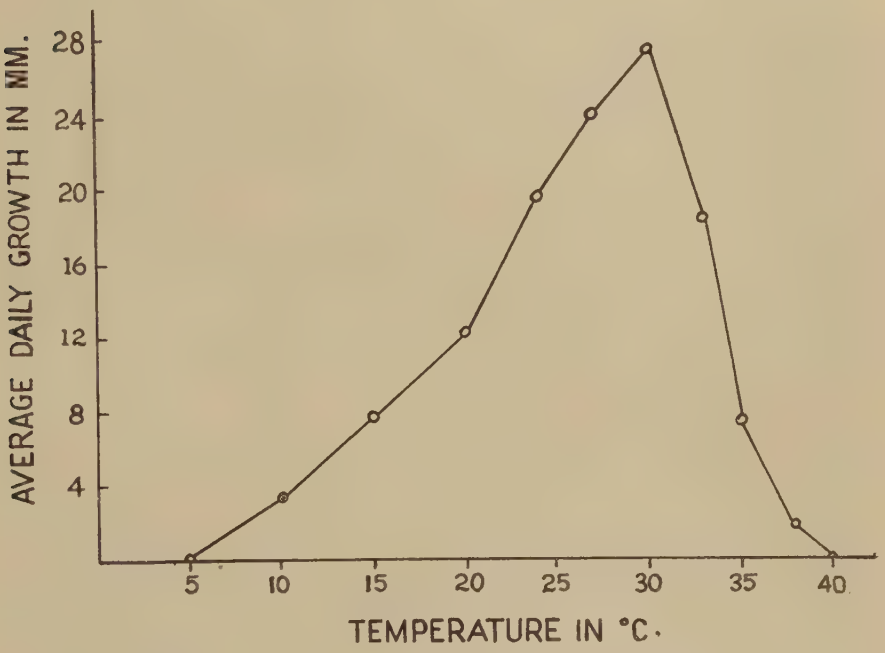

Fig. 1.—Effect of temperature on mycelial growth of *Helminthosporium maydis.*

On the basis of aerial mycelium formation alone, the optimum temperature for growth ranges from 24 to 30°C. Within this range, a balance between aerial mycelium formation and conidial formation seems to be maintained, such that when the fungus produces profuse aerial mycelium it sacrifices the production of conidia. This is evidenced by the fact that there was a scarcity of conidia in the cultures which produced an overabundance of aerial mycelium at 30°C., whereas abundant conidia were present in those cultures at 24 and 27°C. which did not produce aerial mycelium as profusely as those kept at 30°C. Hence, if both mycelial and conidial formation were to be considered, the optimum range of temperature for growth would be from 24 to somewhere below 30°C. and not from 24 to 30°C., inclusive, as mentioned previously.

The maximum temperature for growth was found to be 40°C. Growth at this high temperature was hardly noticeable, having a linear dimension of only 2.4 mm. Even after the period of observation had been extended to more than eight days the growth never increased. It should also be mentioned that out of the eight cultures kept at 40°C., only three showed measurable increments in growth. The rest did not show any sign of growth throughout the experiment.

To determine whether the extremely low and high temperatures (5 and 40°C.) were fatal or not to the fungus, the cultures subjected to these temperatures and in which no growth was observed on the eighth day were kept further in their respective chambers for three weeks. The cultures were then subjected to the favorable temperature (25 to 27°C.) of the laboratory. Since the cultures from the 40°C. chamber had dried up considerably after three weeks, their water content was restored by introducing with a hypodermic syringe enough sterile water into the growth tubes. At the end of two weeks in the laboratory, abundant mycelium and conidia were observed in the cultures which had been kept at 5°C., but none were observed in those previously kept at 40°C. These results indicate that the low temperature merely held back the development of the mycelia and conidia, but the high temperature was fatal to the fungus.

Another interesting point brought out by these experiments relates to the temperature limits of conidial formation and to the effect of temperature on the morphological characteristics of conidia. As mentioned previously, a very scarce development of conidia occurred at 15°C. In the higher temperature range, sporulation ceased altogether at temperatures above 33°C. There was a close parallelism between the number of conidia produced at 15 and at 33°C., it being extremely scarce at both temperatures. The same parallelism occurred at 20 and 30°C., the conidia produced at these temperatures being in approximately equal quantities. Those produced at either 20 or 30°C., however, were not as scarce as those produced at either 15 or 33°C. Conidia were produced in abundance at 24°C. They closely approached in quantity those produced at 27°C., the temperature at which sporulation of the fungus was at its best.

A striking difference was noticed in the shape, septation, and size of conidia which developed at 15, 30, and 33°C. and those which developed at 20, 24, and 27°C. The conidia produced at the latter temperatures were predominantly of the normal type, whereas those produced at 15, 30, and 33°C. were mostly of the aberrant type. Since the characteristics of normal conidia have been described in connection with the morphology of the fungus, only the aberrant characteristics will be mentioned here.

Aberrant conidia were mostly cylindrical or fusiform in shape and did not possess the strong curvature typical of normal conidia. They tapered off from the broad middle cell towards the basal and

apical cells, but to a lesser degree. As a consequence, the basal and apical contours were not as markedly narrow as in the normal type. The basal scar or hilum was generally less conspicuous although many aberrant spores were observed to possess hila as distinct as those of normal ones. The coloration did not vary much from the normal, the spores usually retaining the characteristic fuliginous to olivaceous hue. The characteristics just given generally held true for conidia produced at 15, 30, and 33°C.

As regards septation and size, conidia produced at temperatures too low or too high for optimum growth of the fungus were markedly inferior to those developed at the more favorable temperature of 20 to 27°C. For comparison, the length and width were measured and the number of septa of 100 conidia produced at a given temperature were counted. The results are presented in table 7.

Conidia tended to become shorter, slightly narrower, and less septate as the temperature went below or above the optimum range for growth of the fungus (table 7). Although mycelial growth was not abundant at 20°C., the few conidia produced at this temperature did not deviate widely in size and septation from those produced at 24 and 27°C. which had an abundance of mycelium. In spite of their limited number, the conidia produced at 20°C. still resembled closely those of the normal type in morphological characteristics. In contrast to this, the cultures kept at 30°C., which developed mycelium more profusely than those subjected to 24 and 27° C. and much more so than those placed at 20°C., produced conidia which were generally smaller and less septate than those produced at 20 to 27°C. This fact again seems to support the writer's previous conclusion that a profuse development of mycelium tends to affect adversely the production of conidia. An extremely vigorous vegetative development of the fungus evidently causes a reduction not only in quantity but also in size and septation of the spores.

Effect of temperature on the germination of conidia. The effect of temperature on the germination of conidia was studied by mounting spores, taken from four-week-old corn meal agar slant cultures, in sterile distilled water in van Tieghem cells and incubating them for 12 hours in a series of constant temperature chambers ranging from 5 to 40°C. At the end of the incubation period, the conidia in the van Tieghem cells were examined under the low power of a microscope and the percentage of germination was determined on the basis of 200 spores observed at each temperature. The results are presented in table 8.

The temperature favorable to the germination of conidia obviously lies between 24 and 35°C., 30°C. being apparently the most favorable (table 8). The minimum and maximum temperatures for germination are in the vicinity of 5°C. and 40°C., respectively. It is interesting to note that the temperature which was found to be opti-

mum for mycelial growth and sporulation of *H. maydis* falls within the optimum range for germination of conidia of this fungus.

Thermal death point of conidia. Thermal death point is defined in Dorland's (1947) *American Illustrated Medical Dictionary* as "the degree of heat required to kill young fluid cultures of an organism exposed for ten minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in the thermal water bath." Although the method specified in the definition was originally designed for testing the thermal death point of bacteria, it proved entirely practical for *H. maydis* spores and may prove so for spores of other fungi.

The procedure was as follows: Two thin-walled test tubes of a diameter of 15 mm., each containing 2 ml. of sterile distilled water and held upright by a wooden support, were immersed in a constant temperature water bath equipped with a heating rod, a thermostat, and a stirrer run by an electric motor. The temperature of the water in the test tubes was checked by immersing a thermometer with a centigrade scale into one of the tubes. When the desired temperature had been reached, a drop of heavy spore suspension (spores were taken from 10-day-old corn meal agar slant cultures and suspended in sterile distilled water) was introduced with a medicine dropper into the other test tube. The addition of the tiny drop of spore suspension into the water in the test tube did not cause any noticeable lowering of the temperature of the latter. After a 10-minute exposure of the conidia to a given temperature, the tube was withdrawn from the water bath and its contents distributed with a pipette among several hollow-ground slides. The slides were then placed upon glass-rod supports inside a large Petri dish bottom-lined with moist filter paper and incubated at a temperature of 27°C. The slides were occasionally examined for germinating conidia, and the spore suspensions were prevented from drying out by regular replacement of the amount of water that had evaporated from the slide mounts during the incubation period. Final examination of the slides was made after three days.

The results of this experiment can be summarized as follows: Exposure of conidia to 45-48°C. for 10 minutes gave 10 to 18 per cent germination; exposure to 49-50°C. for the same length of time gave 2 to 5 per cent germination; exposure to 51-51.5°C., 1 per cent germination; and exposure to 52°C., no germination. The experiment was repeated three times and practically the same results were obtained. Since positively no germination was obtained from conidia exposed to 52°C. for 10 minutes, it can be concluded that the thermal death point of conidia of *H. maydis* is at 52°C. or very close to it.

Effect of pH on mycelial growth and sporulation. Like temperature, the pH of culture media frequently limits the vegetative and reproductive activities of many fungi, whether saprophytic or parasitic. In some cases, pH has been demonstrated to influence greatly the ability of pathogenic fungi to cause disease. Thus *Plasmodiophora brassicae*, the cause of the club-root of cabbage, becomes more de-

structive to the host plant when the latter is grown in acid soils (Schaffnit and Meyer-Hermann, 1930; Honig, 1931). On the other hand, *Spongospora subterranea,* the pathogen causing the powdery scab of potato, does more damage when the host is grown in alkaline soils. *Fusarium cubense* has been found to cause a more severe wilting of Manila hemp plants growing in acid soils, whereas in alkaline soils this pathogen is less destructive. Acidity not only enhances the growth of this fungus but also its parasitic activity. Also, under acidic conditions resulting mostly from lack of soil aeration, the host plant becomes more predisposed to the attack of the *Fusarium* because of the retardation in the development of the outer protective tissues of the corms. The fungus, growing more vigorously under conditions detrimental to the host, consequently invades the latter more successfully.

In the present work, the effect of pH in relation to the pathogenicity of *Helminthosporium maydis* was not determined. The writer's experiments were conducted primarily to determine the effect of pH on the mycelial growth and sporulation of this corn pathogen. For this purpose, cultures at different pH conditions were prepared as follows:

To 20 ml. of sterilized corn meal agar contained in a 50-ml. Erlenmeyer flask, varying amounts of either 1 N HCl or 1 N NaOH, which had been previously autoclaved, were added to adjust the reaction of the agar medium to the desired pH, ranging from 1.2 to 11.8. All pH readings were checked by testing samples of the corn meal agar medium, mixed with either acid or alkali, with a Beckman pH-meter. Each of the pH-adjusted media was poured into a sterile 9-cm. diameter Petri plate and allowed to congeal. After 35 hours, each plate was inoculated at the center with a bit of agar medium containing young hyphal tips taken from one-week-old plate cultures of *H. maydis.* The inoculum for each plate was made as uniformly small as possible so that the subsequent growth of the fungus, no matter how slight, could be easily detected and thereby accurately measured. As soon as the plates had been inoculated, they were placed inside incubators which were set at 27°C., the temperature which had previously been found to be favorable for both mycelial growth and sporulation of the fungus. Since all other experimental conditions, with the exception of pH, were held constant, the variations in the amount of mycelial growth and degree of sporulation shown later by the cultures could justifiably be interpreted as having been due to pH effect.

The cultures were taken out of the incubators only for brief periods when growth measurements were made. Since Petri plates instead of growth tubes were employed in these experiments, the amount of growth was based on the diameters of the circular fungous colonies and not on the linear growth dimensions. The diameters of the colonies were measured on the second, fourth, sixth, and eighth days after inoculation. As with the experiments on the effect of

temperature on the fungus, the last growth measurements were made on the eighth day because by that time the cultures under favorable pH conditions had almost covered the entire surface of the agar media. The relative abundance of aerial mycelium and conidia was also finally observed on the eighth day. As in previous experiments, the abundance or scarcity of conidia was determined.

Two series of experiments were conducted at three-week intervals, and in each of the series three cultures at a given pH were observed. The results of the experiments (table 9 and fig. 2) showed

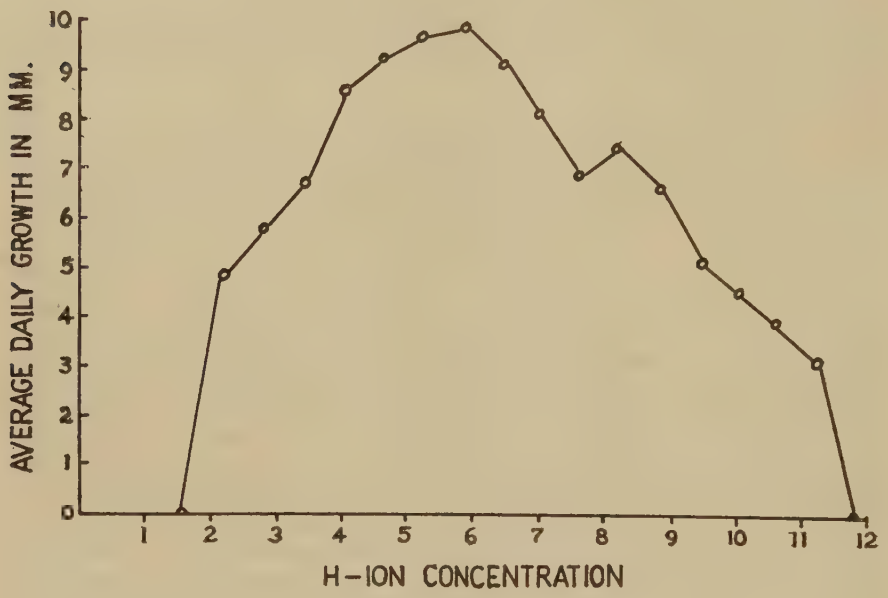

Fig. 2.—Effect of pH on mycelial growth of *Helminthosporium maydis*.

that the minimum pH at which growth of *H. maydis* was possible was somewhere between 1.6 and 2.2. The optimum range for the production of mycelium was from pH 4.0 to 8.2, and the maximum pH was 11.2. The production of both mycelium and conidia was favored by pH ranging from 5.2 to 8.2, and within this range, pH 5.8 and 6.4 were most favorable. The development of mycelium, but not of conidia, was enhanced by pH 4.0. At this pH aerial mycelium was abundant, but conidia were very scarce. Growth of the fungus was depauperate from pH 2.2 to 3.4 and from pH 8.8 to 11.2, the amount of aerial mycelium produced being either scarce or very scarce. Furthermore, the mycelium was characteristically appressed to the culture medium and was lighter in color than that produced under more favorable pH conditions.

Sporulation of the fungus started at pH 3.4 and was inhibited at pH above 10.6. Conidial development was scarce at pH 4.6, 8.8, and

9.4 and very scarce at pH 3.4, 4.0, 10.0, and 10.6. Sporulation was never observed to occur at pH 2.2, 2.8, and 11.2 where mycelial growth was exceedingly poor. It is of interest to note that conidia were produced most abundantly at pH 5.8 and 6.4 where mycelial development was equally abundant.

On the shape of conidia, pH of the culture medium appeared to have no effect at all, in contrast to temperature which had a definite effect. Except in a very few cases, the conidia produced under unfavorable pH conditions were identical in shape with the typical *H. maydis* spores developed under favorable conditions. A difference, however, was noted between the effect of temperature and of pH upon the coloration of conidia. While the conidia produced at unfavorable temperatures retained the typical fuliginous to olivaceous color, those that developed under extremely acidic or alkaline conditions were predominantly gray to very light gray. This decolorizing effect of unfavorable pH was observed not only in the spores but in the mycelium as well.

On the size and septation of conidia, pH seemed to exert an influence similar to that of temperature. Conidia produced under highly acidic or alkaline conditions, that is, pH conditions below and above the optimum range, were small and had few septa, like the conidia produced at low and high temperatures.

A comparison of cultures growing in Petri plates and in growth tubes revealed that the rate of growth of the latter was more than twice that of the former. The average rate of growth of plate cultures at pH 5.8 and 27°C. was 9.88 mm. (table 9); that of growth-tube cultures subjected to the same temperature and approximately the same pH was 24.06 mm. (table 6).

That the fungus could adapt itself better to increasing pH than to increasing temperatures is evidenced by the abrupt ascent and gradual descent of the curve shown in figure 2 and the slow rise and rapid descent of the curve in figure 1. It can be deduced that *H. maydis* can better withstand the changes in pH than the changes in temperature as long as either changes are not too far above the optimum range.

Studies on pathogenicity

Inoculation experiments on maize plants of different ages with four isolates of the pathogen. Two varieties of corn, namely Golden Bantam (a sweet corn) and Sweepstakes (a field corn), purchased from the Perry Seed Company in Boston, Massachusetts, were used in artificial inoculation tests. Seeds of these two varieties were disinfected with 1:1000 mercuric bichloride solution for 10 minutes, washed in three changes of sterile distilled water, and soaked in sterile water overnight. They were planted in sterile potted soil the following day, five to eight seeds in each six-inch pot. The plants that subsequently developed were used in the inoculation tests. The

youngest plants used were 3 to 4 inches high with 2 to 3 leaves; the oldest were 10 to 15 inches high with 5 to 8 leaves.

The inoculum consisted of conidia suspended in sterile distilled water. Conidia from two-week-old potato-dextrose agar plate cultures were scraped off and placed in tubes of sterile distilled water. The suspension was passed through cheesecloth to remove as much as possible of the mycelial fragments and conidiophores that were scraped off with the conidia. The inoculum was sprayed by means of a sterile DeVilbiss atomizer on the aerial parts of the plants.

The inoculated plants were mostly incubated for 36 hours under bell jars immediately after inoculation. Control plants, comparable in age, height, and vigor to those inoculated, were sprayed with sterile distilled water alone and also placed under bell jars for the same length of time. After the incubation period, the bell jars were removed and the plants were left on benches in the greenhouse for further observation.

In a few experiments, the bell jars were dispensed with and both the inoculated and control plants were left uncovered. Under these conditions, the inoculated plants also developed infection, but the appearance of the symptoms was much delayed and the degree of infection was greatly reduced. On an inoculated plant incubated under a bell jar, the earliest symptoms of the disease were discernible 24 hours after the application of the inoculum, and as many as 50 or more water-soaked lesions could be counted on a leaf about twelve inches long and three-quarters of an inch wide at the middle. On the other hand, a comparable plant, inoculated but not covered with a bell jar, did not show distinct signs of infection until 40 to 48 hours after application of the inoculum, and a leaf of the same dimensions as mentioned previously merely showed young spots that rarely exceeded 30 in number.

The two varieties of corn used in these inoculation experiments showed identical reaction to the four isolates of *Helminthosporium maydis*. Infection was invariably evidenced by the appearance of numerous water-soaked spots ranging from 1.0 to 2.5 mm. in diameter. On plants incubated under bell jars, the spots were very noticeable 36 hours after inoculation. They were more or less transparent especially on young and tender leaves. Viewed against the light, the individual spots could be seen to have at the center a minute speck slightly lighter in color than the surrounding tissues. The speck marked the point of penetration of the germ tube as shown by leaf sections under the microscope. Very young plants, particularly those with two to three leaves, withered completely in three to five days when heavily infected. In older plants, portions of the leaves with numerous infections rapidly became pale green, then yellow, and withered in 10 to 15 days. Leaves with moderate infections

showed partial withering only in the regions immediately bordering the lesions.

After every inoculation test, the pathogen was reisolated from the inoculated plant as soon as typical symptoms of the disease developed. The reisolated fungus was compared with the original stock cultures. In all instances the pathogen was recovered from the infected corn plants. Subsequent inoculation tests proved that the reisolated cultures were just as pathogenic as the parent cultures. The identity of the reisolated fungus was further confirmed after portions of the diseased host tissues were incubated in large Petri dishes lined inside with moist filter paper. Within a week, the fungus invariably grew and sporulated profusely from the incubated diseased tissues.

From March, 1947, to April, 1949, a total of 12 inoculation experiments was carried out with maize plants of different ages. In the first five experiments, the Philippine and Pennsylvania isolates were used as inoculum. The ages of the inoculated plants ranged from 10 to 40 days. In the next three experiments, the Beltsville isolate was tried in addition to the Philippine and Pennsylvania isolates. The inoculated plants were from 25 to 45 days old. In the last four experiments, plants ranging in age from 10 to 40 days were inoculated with the Beltsville and Cuban isolates.

The results of the experiments showed that the four isolates of *Helminthosporium maydis* were able to cause 100 per cent infection on both sweet and field corn ranging in age from 10 to 45 days (table 10). If the virulence of the four isolates were to be judged on the basis of percentage of infection alone, all four isolates could immediately be concluded to be equally virulent. Yet such a conclusion would be erroneous as the severity of infection differed among the inoculated plants of different ages. A difference was also noted when plants of the same age were inoculated with any of the four isolates. In view of these observations, the infected plants were classified under three categories, namely, heavy, moderate, and light infections. Infection was arbitrarily designated as heavy when more than half of the foliage area of the plant was diseased; moderate, when one-half of the foliage area was involved; and light, when less than half showed the disease. In every instance, the infected plant was given its proper designation one week after it was inoculated. After the infected plants had been grouped under the three categories, the rating of infection for each experiment was computed according to the following formula devised by Nisikado and Miyake (1926):

$$\frac{H \times 3 + M \times 2 + L \times 1}{\text{No. of inoculated plants} \times 3} \times 100 = \text{Rating of infection}$$

where H = number of heavily infected plants
M = number of moderately infected plants
L = number of slightly infected plants.

The ratings of infection of the two varieties of maize at different ages (table 10 and figs. 3 and 4) showed that the Pennsylvania

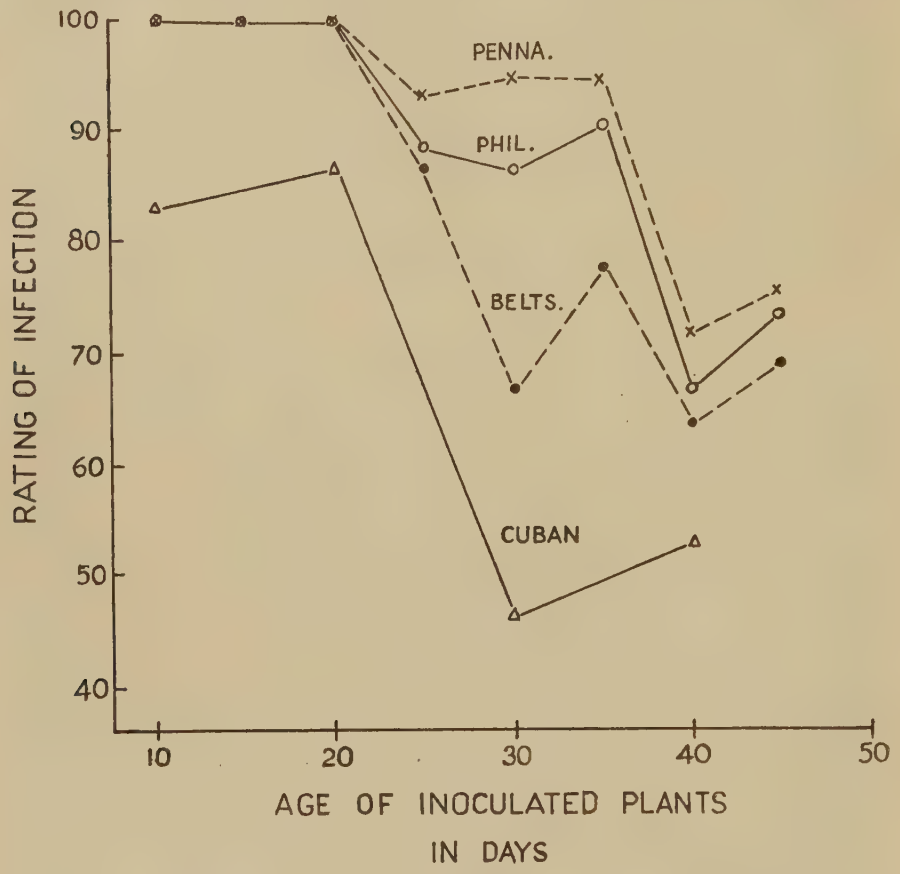

Fig. 3.—Comparative pathogenicity of four isolates of *Helminthosporium maydis* on sweet corn var. Golden Bantam at different ages.

isolate of *H. maydis* is more virulent than the other three. The greater virulence of this isolate was more apparent on plants more than 20 days old since on these plants the rating of infection for this isolate remained relatively high, whereas the ratings for the other three isolates were markedly low. For instance, on 30-day-old maize plants of the variety Golden Bantam at their fruiting stage were used Pennsylvania isolate was 94.44; for the Philippine isolate, 86.66; for the Beltsville isolate, 66.66; and for the Cuban isolate, 46.66.

The same trend was noted in the 30-day-old plants of the variety Sweepstakes. If, in addition to these figures, the average infection ratings were considered, it would seem justifiable to conclude that the four isolates are not equally virulent and that in point of pathogenicity they rank according to the following order: first, Pennsylvania; second, Philippine; third, Beltsville; and fourth, Cuban isolate.

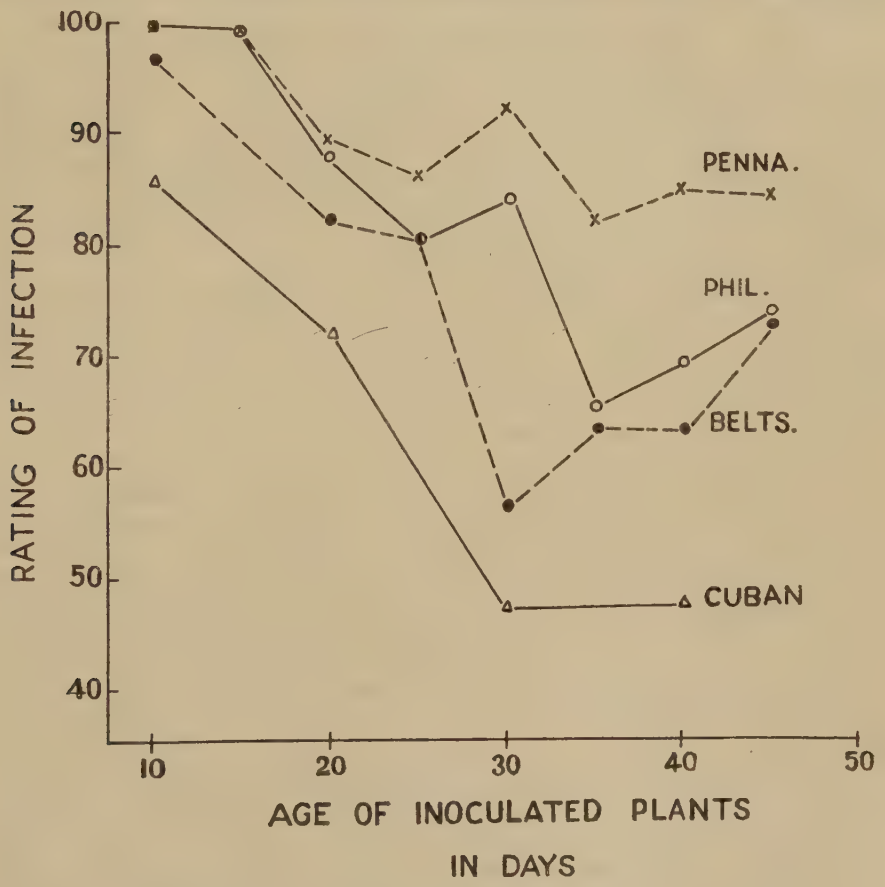

Fig. 4.—Comparative pathogenicity of four isolates of *Helminthosporium maydis* on field corn var. Sweepstakes at different ages.

Inoculation of other aerial parts of the maize plant. To test the pathogenicity of *H. maydis* on the various plant parts, the writer conducted inoculation experiments in the greenhouse. Sweet corn plants of the variety Golden Bantam at their fruiting stage were used in the experiments, and spore suspension of the Pennsylvania isolate was used as inoculum. The effects of injury and humidity on the inoculated parts were also observed. When the parts to be inoculated had to be injured, tiny superficial incisions about 5 mm. long

were made with a sterile scalpel. The inoculated parts were kept moist by wrapping them with four layers of wet cheesecloth during the incubation period which usually lasted for a week. The pathogenicity of the fungus was also tested on the tassels. The tassels were enclosed in an oiled paper bag with or without a wet wad of cotton inside. The results of the experiments are summarized in table 11.

Uninjured stems and mature midribs, whether subjected to humid conditions or not, were not attacked by the fungus (table 11). These plant parts, however, became infected when injured and subjected to humid conditions, but not when they were free from moisture. In contrast, the uninjured leaf sheaths and bracts of ears were readily infected by the fungus, whether or not they were kept moist. They developed symptoms resembling those typically formed on the leaf blades. The tassels did not become infected regardless of whether they were placed under humid conditions or not.

Soil-inoculation experiments. *Helminthosporium maydis* is primarily a foliar parasite; the writer has never observed it to infect maize roots in the field. A case, however, has been reported in which this fungus was able to cause maize root rot both in the field and in the laboratory (Richardson, 1942). To test whether the isolates of *H. maydis* under study could infect the roots, the writer conducted soil-inoculation experiments.

The Pennsylvania, Philippine, Beltsville, and Cuban isolates of the fungus were grown in a corn meal-sand medium prepared according to the formula given by Riker and Riker (1936). The medium, which consisted of a mixture of 1000 gm. corn meal, 1000 gm. white washed sand, and 1500 ml. distilled water, was placed in one-quart fruit jars which were plugged with cotton and autoclaved for two hours for two consecutive days. The jars, which were half-filled with the medium, were seeded with 5 ml. of a heavy spore suspension of the fungus and incubated in the laboratory. After a four-week incubation period had resulted in a heavy sporulating growth of the fungus, the substratum, together with the fungus, was ground finely with a mortar and pestle and then mixed with sterilized potted soil in the proportion of one part by volume of inoculum to nine parts by volume of the soil. As control, sterilized corn meal-sand medium was mixed with sterilized soil in the same proportion also. Ten maize seeds of the variety Golden Bantam, previously disinfected with 1:1000 mercuric bichloride solution and thoroughly washed with sterile water, were planted in each inoculated and noninoculated pot of soil. Additional controls were prepared by planting disinfected seeds in sterilized soil to which no medium had been added. All pots were placed on a bench in the greenhouse, watered regularly, and observed for germination of seeds and appearance of the disease in the seedlings. Roots were examined for the presence of lesions one month after the seedlings had emerged from the soil. The results of the inoculation experiments are presented in table 12.

Planting seeds in soil inoculated with *H. maydis* resulted in a decrease in the percentage of germination (table 12). Examination of the ungerminated seeds usually showed that the scutellum and endosperm were infected with the fungus. Seeds that managed to germinate frequently developed infections on the radicle and coleoptile, and consequently the young plants failed to survive. The plants that escaped infection at emergence became stunted and generally unhealthy in appearance. The roots of these plants, in most instances, showed lesions of the disease, the tips of the main as well as lateral roots being usually involved and the infected roots assuming a dark brown to black coloration. When infection occurred much later in the development of the roots, the functions of the latter were only slightly impaired and the aerial parts of the plants grew normally. The foregoing observations seem to indicate that the fungus in the soil was most destructive from the time the plants were just beginning to emerge until they were 2 to 3 inches above the soil level. Root and coleoptile infections developed during this period invariably resulted in a severe root rot and a consequent collapse of the aerial plant parts. In conclusion, the positive results of soil-inoculation experiments in the greenhouse indicate that under favorable conditions in fields where an abundance of infected maize tissues has been left after harvest, the fungus may be reckoned with as a possible cause of root rot of succeeding maize crops.

Cross-inoculation experiments. The facility with which conidia of *Helminthosporium maydis* are disseminated by wind, rain, insects, and other agents suggested the possibility that the conidia thus dispersed might be able to cause infection on other grasses. Once infected, these grasses could provide a means for the fungus to tide over the seasons when maize is absent in the field. Thus they would serve as source of inoculum for newly planted maize plants. Faced with this possibility, the writer conducted cross-inoculation experiments to determine what species of grasses other than maize can be parasitized by *H. maydis*.

A part of the cross-inoculation work reported here was previously performed by the writer at the College of Agriculture, Los Baños, Laguna, in 1944. This work was augmented by experiments done in the greenhouse of the Biological Laboratories at Harvard University in the summer and fall of 1947 and at the Atkins Garden, Soledad, Cuba, in the summer of 1948.

In all the cross-inoculation tests conducted in the Philippines, Harvard, and Cuba, different species of Gramineae were grown in loam soil contained in earthen pots. The plants were potted because conditions could not be controlled adequately in the field. The plants were started from one of three types of propagating materials—seeds, tillers, or stem cuttings—depending on which type of material was most available and best suited for the rapid propagation of each species. After a month or so, when the leaves were well developed, they were sprayed by means of a DeVilbiss hand atomizer

with conidia taken from a three-week-old potato-dextrose agar culture of *H. maydis* and suspended in sterile distilled water. In every experiment the plants were sprayed again with the spore suspension after 24 hours to make doubly sure that the inoculum was adequate. The inoculated plants were incubated in damp chambers for 48 hours. When the plants like rice and sugar cane showed no ill effects from the 48-hour exposure in damp chambers, the incubation period was prolonged for 24 hours more. The plants were finally moved out of the damp chambers and placed in the shade for further observation. Control plants sprayed with sterile distilled water and also placed in damp chambers were maintained in all experiments.

The results of the experiments showed that of the 28 species belonging to 21 genera of grasses inoculated, five species belonging to four genera showed lesions from which the fungus was successfully reisolated (table 13). No infection was obtained from the other grasses. Repeated inoculations of these apparently resistant species consistently yielded negative results.

The five species of plants which were successfully inoculated with *H. maydis* belong to two closely allied tribes of the family Gramineae. *Rottboellia exaltata* and *Saccharum officinarum* belong to the tribe Andropogoneae and *Euchlaena mexicana, Tripsacum latifolium,* and *T. laxum,* to the tribe Tripsaceae. From the phylogenetic point of view, it seems significant that members of these tribes reacted to a pathogen of maize, another member of the tribe Tripsaceae. The fact that all these grasses showed an inherent susceptibility to one pathogen perhaps further justifies their being placed in their present taxonomic positions. Further proof of the close relationship existing among these grasses was shown by Weston (1920) who found that *Sclerospora philippinensis,* the cause of the downy mildew of maize in the Philippines, could also infect teosinte and sorghum which belong to the maize tribes Tripsaceae and Andropogoneae, respectively.

Of the five species which were infected with *H. maydis,* teosinte (*Euchlaena mexicana*) was the most susceptible. The symptoms on the leaves markedly resembled those produced on maize leaves particularly during the early stages of infection. The shape and coloration of mature lesions approached closely those of similarly mature lesions on maize leaves.

Rottboellia exaltata appeared to be less susceptible to the fungus than teosinte. The progress of infection in this plant was not as rapid as that in teosinte. Relatively fewer lesions were formed which, moreover, were slightly inferior in size to those formed on teosinte.

Sugar cane (*Saccharum officinarum*) and the two species of gama grass (*Tripsacum latifolium* and *T. laxum*) showed identical reactions to the fungus. They developed lesions consisting of minute, dark-brown specks which were sparsely distributed on the leaves. These specks did not increase in size nor in number despite the presence of favorable conditions for the development of the pathogen.

In contrast to the results of the writer's tests, Drechsler (1925a & b) reported that incipient lesions were produced on rice (*Oryza sativa*) inoculated with *H. maydis*. Nisikado and Miyake (1926), working on the same fungus, obtained positive results from inoculations on millet (*Setaria italica*). Although these plants were tested in the writer's cross-inoculation work, not even the slightest evidence of infection was obtained. Since the physical conditions under which the writer conducted his experiments were identical with those described by the former investigators, the discrepancy in results could perhaps be attributed to the difference between the strains of the fungus and the varieties of the plants used by the former workers and those used by the present writer.

The pronounced pathogenicity of *H. maydis* on teosinte, resulting in the production of leaf lesions which bear a remarkable similarity to the lesions on maize leaves, seems to lend support to Drechsler's (1925a &b) conclusion that the species of *Helminthosporium* which he found naturally occurring on teosinte is identical with the maize parasite. Drechsler did not inoculate maize with the teosinte fungus; neither did he inoculate teosinte with the maize fungus. His conclusion was based mainly on his observation of the close similarity in morphology and pathological habit between the parasites of the two host plants. On account of the close relationship between maize and teosinte, however, his conclusion might not be too remote from reality.

It can be concluded that at least two of the species of Gramineae tested by the writer—*Euchlaena mexicana* and *Rottboellia exaltata*—can serve as suitable wild hosts of *H. maydis*. The fact that these two species thrive in tropical maize-growing regions and occur as common weeds in the field presents a problem in the prevention and control of the leaf spot of maize caused by *H. maydis*. Since these two plants serve as potential source of inoculum for maize, their eradication, as far as practicable, from maize fields and adjacent areas is recommended.

The results of the artificial cross-inoculations on sugar cane, *Tripsacum latifolium,* and *T. laxum* indicate that these plants probably can not be infected by the maize pathogen under natural conditions. It is unlikely, therefore, that their cultivation near maize fields will be inimical to the maize plants.

Pathological histology

The progress of the disease in the host plant was observed by means of suitable histological techniques from the time the plant became infected by the fungus until it showed the typical symptoms of the disease. Observations were made on the mode of penetration of the pathogen, the subsequent development of the hyphae within the host tissue, and the effect of the parasite on the walls and contents of host cells.

Mode of penetration. Thirty- to 45-day-old healthy maize plants were inoculated in the usual manner with a spore suspension of *H. maydis* and incubated under bell jars for 36 hours. Some of the plants were injured by puncturing the leaves with a sterile needle before inoculation. Sections of fresh infected leaves were prepared at 12-hour intervals from the time of application of the inoculum until the fungus had firmly established itself within the host tissues. The leaf sections were stained with Pianeze III B according to the method given by Vaughan (1914). Satisfactory results were obtained with this stain, for it gave good differentiation of mycelium within the host tissue, the reddish mycelium being easily distinguished from the light-green host tissue. The preparation of the sections at intervals of 12 hours after inoculation enabled the writer to follow the sequence of development of the invading fungus and at the same time observe the changes undergone by the host tissues as a result of the parasitic activity of the fungus.

From the sections that were observed, it was found that the germ tube, which was extruded from either the basal or the apical segment of a conidium, penetrated the leaf either through the stomata, through injuries, or directly through the epidermis (Pl. III, figs. 10 and 11).

The length attained by a germ tube before it is able to effect any penetration varies (table 14). In some instances, penetration occurs after the tube has attained a length approximately equal to that of the conidium producing it. In others, the infection tube elongates to 200 or more microns prior to penetration—a dimension almost three or more times that of the conidium from which the tube originates.

An interesting feature of the germ tube is the ovoid to spherical swelling formed at the point of penetration on the host leaf (Pl. III, fig. 11). Its diameter ranges from approximately twice to as much as three times that of the tube. Evidently the infection hypha ensues from this swelling and eventually forces itself into the mesophyll of the leaf. The swelling is invariably formed by the germ tube whether penetration is through stomata, through injuries, or directly through the epidermis.

Nisikado and Miyake (1926) and Yu (1933) reported the formation of appressoria at the tips of the germ tubes of conidia of *H. maydis.* They described the appressoria as variously shaped—globular, ovoid, or reniform. In the present work, the writer never observed at the tips of the germ tubes any elaborately shaped structures which would approach in character the elaborate appressoria noted by the Japanese and Chinese investigators. The swellings, however, which the writer observed resembled closely the simple globular and ovoid appressoria described by these investigators. In the opinion of the writer, these hyphal swellings should be regarded as simple types of appressoria since they evidently serve

the same purpose as the elaborate types of appressoria reported by the previous workers.

Table 14 gives an idea of the variation in length of germ tubes at the time of penetration, or until appressoria are formed at their tips. Table 14 also shows the frequency of occurrence of stomatal and direct epidermal penetrations. The infected leaf sections, from which the data presented were obtained, were prepared 48 hours after inoculation of uninjured plants.

The presence of stomata does not seem particularly necessary for successful penetration of the germ tubes since direct epidermal penetration occurred more frequently than stomatal penetration (table 14). Moreover, the wirter observed many cases in which the germ tubes continued to elongate across a stomatal pore or directly beside it without penetrating through it (Pl. III, fig. 10). Penetration in such cases was found to have taken place at a considerable distance beyond the stomatal pore. The role of stomata, however, in the initiation of the disease is definitely significant since these natural openings undoubtedly provide vulnerable points of entry for the pathogen. There is also no doubt that mechanical injuries, such as punctures made by leaf-sucking insects, provide suitable points of entry and that they make no little contribution to the aggravation of the disease. This assumption is confirmed by the observation that leaves with numerous needle punctures were invariably more severely spotted than leaves which were not wounded before inoculation.

Mode of tissue invasion. Twenty-four hours after the infection hypha has penetrated the epidermis of the leaf, a minute water-soaked lesion becomes visible on the leaf. Sections through such lesions often reveal that the infection hypha has progressed down into the mesophyll and has branched out to produce hyphae which are intracellularly located. The mesophyll cells which are invaded by the hyphae often appear slightly shrunken at the beginning. As the disease progresses, the cell contents become disorganized and the cell walls gradually become discolored. At a later stage of the disease the cell walls assume a dark-brown color. A noticeable change also occurs in the cells adjacent to the invaded cells. They too show some degree of shrinking and the cell walls become discolored, but not to the same extent as the walls of the invaded cells. These changes seem to occur in advance of the invading hyphae. It is possible that the contents of these neighboring cells diffuse through the walls of the cells where the hyphae are actually present and thereby provide additional nourishment for the actively growing fungus. It is also possible that in the course of its growth, the fungus excretes or elaborates an injurious substance which causes the eventual death of the cells and the discoloration of the walls. Evidence supporting this contention has been obtained and is to be discussed in paper II.

Since the fungus is a facultative parasite, the collapse and death of the host cells, even before they are actually invaded, would in no

way hinder the fungus from growing further. Thus the hyphae which have originated from two or more infection threads continue to advance towards each other following the longitudinal axis of the leaf and in this way cause the end-to-end coalescence of formerly distinct and separate lesions. The hyphae also advance transversely from either the upper or the lower epidermis, depending on where the inoculum was applied, to the opposite epidermis, thus resulting in the production of lesions which are visible on both surfaces of the leaf. This transverse invasion is facilitated by the thinness of the host leaf.

Occasionally the hyphae are observed to invade the relatively thicker-walled cells of the vascular bundles, but by and large their attack is restricted to the more delicate and thinner-walled cells of the mesophyll. The larger veins of the leaf seem to be able to resist the hyphal invasion to some extent, but at times they also succumb to the attack of the fungus. The resistance offered by the thick-walled vein elements prevents, in many instances, the side-to-side coalescence of lesions. This explains the occurrence on the infected leaf of numerous lesions which are confined to a single intervascular region.

A young and tender leaf, when heavily infected, does not remain turgid and functional for long. Within four or five days, especially when conditions have been favorable for the growth of the fungus, the leaf assumes a blighted appearance, hangs on its own weight at the point of junction to the leaf sheath, and eventually perishes. When infection occurs on an older and more fully developed leaf, the disease progresses less rapidly and impairment of the function of the leaf takes a slower course. If the infection is heavy, numerous necrotic areas appear on the leaf resulting in a tremendous reduction of the photosynthetic area. Furthermore, the translocation of water and raw materials for photosynthesis is greatly interfered with, possibly by the successful fungous invasion of the smaller vascular bundles and by the more extensive destruction of the mesopyll cells. Consequently the whole leaf is starved, and death of the cells which make up the remaining green patches of the leaf gradually ensues.

Under natural conditions, a mature but severely spotted leaf may remain rigidly attached to the stem for some time, but with high humidity and temperature such as those obtaining in artificial inoculation experiments inside damp chambers, the infected leaf may break at any point along the lower half of the blade or usually at its junction to the leaf sheath. It then remains hanging at the side of the stem.

When conditions are favorable for sporulation of the pathogen, the mycelium lying dormant within the necrotic leaf tissues is activated. The fungus resumes growth and hyphae are pushed out either directly through the epidermis or through the stomata. These hyphae, emerging singly or in tufts from the epidermis or stomata, are

differentiated from the parent mycelium and become the conidiophores from which crops of conidia are produced. Upon maturity, the conidia become detached from the conidiophores and are dispersed by various agents. Thus they provide secondary inoculum which can either reinfect the same plant or infect both neighboring and distantly located host plants.

LIFE HISTORY OF HELMINTHOSPORIUM MAYDIS IN RELATION TO PATHOGENESIS

Previous observations in fields in the vicinity of the College of Agriculture at Los Baños which had been planted to maize successively for two years (two crops a year) showed that the leaf spot appeared from season to season. The disease had been observed on both dry- and wet-season crops, infection being invariably more severe on the latter. The question arose, therefore, as to what form or forms enable the fungus to survive from season to season and how long the fungus can live in the absence of living host plants in the field.

The ascigerous stage of *Helminthosporium maydis* has never been observed in the field. The fungus also never produces resistant sclerotial bodies such as those produced by *Helminthosporium sigmoideum,* the cause of a stem rot of rice (Tisdale, 1921; Tullis, 1933a, 1933b; Tullis and Cralley, 1941). Excluding the wild hosts on which the fungus might live over, the only possibility for the fungus to live from season to season seems to be through mycelium and conidia on infected maize residues left in the field after harvest. It seemed desirable then to test the longevity of mycelium and conidia of *H. maydis* produced on host tissues; hence, the following experiments were conducted in the laboratory and in the greenhouse.

Longevity of the mycelium

On March 15, 1947, one-month-old potted corn seedlings of sweet corn variety Golden Bantam were inoculated with a heavy spore suspension taken from three-week-old potato-dextrose agar cultures of the Philippine isolate of *Helminthosporium maydis.* A total of 50 plants was inoculated. All of the inoculated plants were severely spotted and seven of them died in the second week. One month after inoculation, all the leaves from the badly diseased and the dead plants were removed, air-dried in the laboratory, and finally incorporated with sterile soil in pots in the greenhouse. The 10 pots were divided into two groups. The five pots in the first group were kept dry throughout the experiment; those in the second group were watered every other day so that the soil was always moist. From these pots of sterile soil mixed with infected leaves, leaf fragments were taken at one-month intervals and tested for the viability of mycelium.

The leaf fragments were thoroughly washed with sterile distilled water to remove the soil particles and conidia that might have been

produced. They were then cut into sections of about 3 mm. sq., surface-sterilized in 1:1000 mercuric bichloride solution, washed in three changes of sterile distilled water, and plated out (four sections in one Petri dish) on sterile potato-dextrose agar. The plates were then incubated at room temperature for 72 hours. This length of incubation period was found to be ideal for these experiments because after this time, if the mycelium within the leaf sections were still viable, growth was already fairly advanced and usually young conidia had already been produced.

The results of the experiments showed that *H. maydis* is able to persist in the form of dormant mycelium within the host tissues in the soil for as long as 20 months, provided the soil remains relatively dry (fig. 5). Moisture causes a more rapid decline in viability of

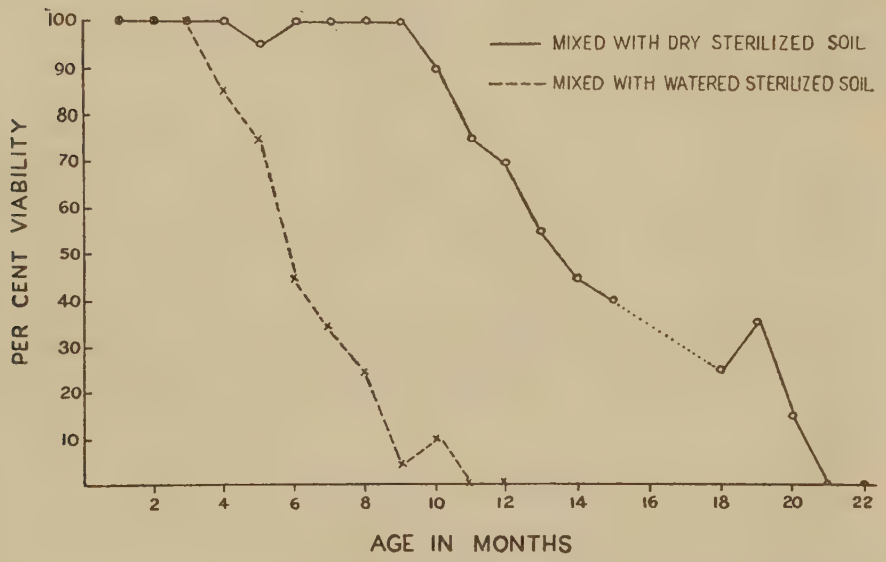

Fig. 5.—Viability of mycelium of *Helminthosporium maydis* within host tissues mixed with dry sterilized soil and with watered sterilized soil.

the mycelium, as shown by the fact that the infected leaves, incorporated with soil which had been kept constantly moist, failed to give rise to any growth of the fungus within the tissues after 11 months. In contrast to this, 75 per cent of the sections taken from infected leaves incorporated with the dry soil contained viable mycelium after the same length of time.

The writer's results corroborate those obtained by Drechsler (1925a & b) who found that old conidiophores and conidia taken from diseased material collected in November, 1921, and stored in the laboratory until October, 1924, (a period of 36 months) could in no

way be made to germinate. The same material, however, when placed in a damp chamber, gave rise to fresh fructifications. This fact, Drechsler concludes, is indicative of the considerable measure of longevity of the mycelium of the fungus found within the tissues of the diseased leaves.

Similar cases demonstrating the remarkable longevity of the mycelium of other species of *Helminthosporium* have been reported by different workers. Paxton (1922) found that the mycelium in herbarium specimens of barley infected with *H. gramineum* remained viable for 16 years. Two weeks after the 16-year-old specimens had been placed in a moist chamber, fresh conidiophores and conidia were produced from the dormant mycelium. Christensen (1922) observed that *H. sativum* could overwinter as mycelium in the seed and on plant remains in the field. He further found that the mycelium in the seed remained viable for several years and that in the laboratory it stayed alive after having been buried in coarse moist sand for more than five months.

From the over-all results of the writer's experiments, it can be deduced that infected maize residues left in the field after the season's harvest serve as source of inoculum for primary infection of the following maize crop which, as commonly practiced in the Philippines and perhaps in some other maize-growing countries, is planted three to four months after the preceding crop.

Longevity of the conidia

As pointed out before, it is possible that the conidia of *H. maydis* are also capable of tiding the fungus over from season to season. To test this possibility, two sets of experiments were carried out to determine the viability of conidia of the fungus at different ages.

The conidia used in the two sets of experiments were taken from two separate lots of infected maize leaves. The first lot consisted of diseased materials which were stored inside covered glass jars in the laboratory throughout the experiments. These specimens, which had an abundance of conidia, were collected in the College of Agriculture at Los Baños on November 7, 1946, and were received by the writer on December 26, 1946. The second lot consisted of diseased maize leaves (artificially inoculated) which were incorporated with dry, potted, sterilized soil in the greenhouse. The same diseased leaves were concurrently used for testing the longevity of mycelium of the pathogen.

Conidia were scraped off lightly from the surface of the lesions, suspended in sterile distilled water, and centrifuged for three minutes. The resulting spore suspension was used in determining the viability of the conidia. Hanging drops in van Tieghem cells were prepared from the spore suspension and placed in an incubator for 12 hours at 27°C. After this time, the hanging drops were examined

under the microscope, and records of the germination of conidia were obtained.

It was assumed that conidia which failed to germinate, or at least did not show any visible sign of producing a germ tube after 12 hours in the incubator, were no longer viable. If they were viable at all, conidia ordinarily commenced to germinate within two hours, although the majority of them did not start to do so until after the third hour. Moreover, numerous observations showed that under the conditions of the viability tests, conidia which remained ungerminated after 12 hours never germinated at all even when the period of incubation was extended to 24 hours.

On account of the scarcity of conidia produced on the second lot of diseased materials, i.e., the lot stored in the greenhouse, it was deemed practicable to observe the viability of conidia at intervals of two months rather than at the usual monthly intervals as in the first lot.

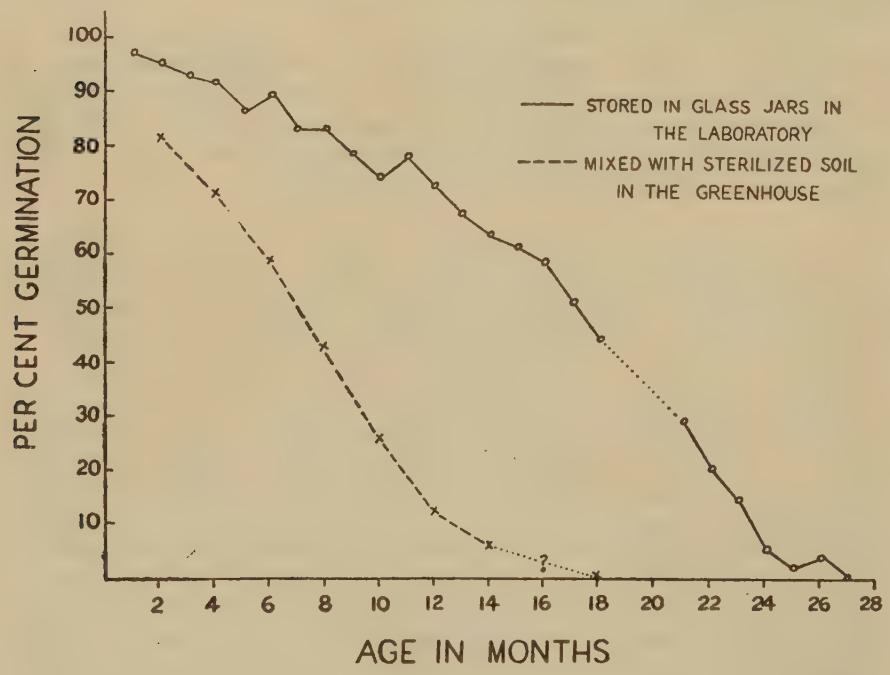

Fig. 6.—Viability of conidia of *Helminthosporium maydis* stored in glass jars in the laboratory and mixed with sterilized soil in the greenhouse.

The results of the experiments showed that in the laboratory, conidia of *H. maydis* are able to retain their viability for a relatively long time, since at the age of 17 months more than half the conidia tested were still able to germinate (fig. 6). A more or less rapid de-

cline in viability was noticed after the eighteenth month, and on the twenty-seventh month not one of the 182 conidia tested germinated, indicating a complete loss of viability. Germination experiments were repeated on the twenty-eighth month, but the results were all negative.

Mixture with soil and exposure in the greenhouse seemed to have had an adverse effect effect on the viability of conidia. On the fourteenth month, conidia kept in the laboratory gave 63.28 per cent germination; those kept in the greenhouse gave only 6.86 per cent. Furthermore, on the eighteenth month, all conidia from the greenhouse failed to germinate, whereas those from the laboratory still gave 44.83 per cent germination.

Perhaps the difference in viability between the two lots of conidia can be attributed partly to the difference in temperature in the greenhouse and in the laboratory. During the winter months of 1947 to 1948, the conidia in the greenhouse had been subjected to temperatures which fluctuated from 16°C. at night to 22°C. at midday. In the spring and autumn, the fluctuations in temperature were not as great as those in winter, the range being approximately between 2° above and below 21°C. In contrast to these varying temperatures in the greenhouse, the temperature in the laboratory during the autumn, winter, and spring months remained more or less constant between 24° and 25°C. In summer, the greenhouse and laboratory temperatures were closely comparable except on extremely hot and sunny days when the former exceeded the latter by 2 to 3°C.

In addition to temperature, moisture was also possibly a contributive factor to the great decline in viability of the conidia in the greenhouse lot. The potted soil with which the conidia were incorporated had been dampened to some extent by occasional water splashes when nearby potted plants had been watered with a rubber hose. On the other hand, the conidia in the laboratory had always been kept in a moisture-free atmosphere. It thus seems logical to suppose that alternate drying and moistening of the conidia appreciably lessened their viability.

That temperature and moisture are instrumental in either shortening or prolonging the viability of conidia was demonstrated by Page, Sherf, and Morgan (1947). These investigators showed that with controlled relative humidity, conidia of *Helminthosporium oryzae* retained their viability much longer when stored at low temperatures. They also found that relative humidity during storage had a more important influence on longevity than temperature alone. They showed that the lower the relative humidity, the longer the conidia remained viable regardless of temperature (minimum of 10°C. and maximum of 31°C.). With increased relative humidity, however, only the conidia stored at low temperatures retained their viability for a long period; those subjected to higher temperatures lost their viability rapidly.

The effects of temperature and humidity on the viability of rust and smut spores have also been investigated by Peltier (1925) and Noble (1934), repectively. Peltier found that the lower the temperature, the longer the urediospores of *Puccinia graminis tritici* retained their viability at all relative humidities. Noble showed that spores of *Urocystis tritici*, the cause of flag smut of wheat, retained their viability for at least ten years when stored at relative humidities below 33.5 per cent at uncontrolled temperatures which ranged from 13 to 31°C.

Raper and Alexander (1945) conducted extensive lyophilization experiments with their large collection of Aspergilli and Penicillia and with some members of the Mucorales, Entomophthorales, and Hyphomycetes. Among the Hyphomycetes was a species of *Helminthosporium* (NRRL No. 1680) which was given a rating of "fair viability" 19 months after it had been lyophilized. If the survival from the rigors of the lyophilization process and the retention of viability for a relatively long period by the conidia of this one species of *Helminthosporium* are true of the other members of the genus, it is very likely that conidia of *H. maydis* will be able to live in the field even in the absence of living host plants for a few months at least, provided that the relative humidity remains at a low level.

The likelihood of conidia of *H. maydis* surviving for several months in the field is borne out by the results of the writer's test on the viability of coniddia exposed in the field. In this test, conidia were obtained from infected maize leaves sent to the writer by Dr. C. H. Arndt from Clemson, South Carolina. Of 300 conidia observed, 57, or 19 per cent, were viable. The fact that these conidia, which had been exposed in the field in Clemson for approximately seven months (late summer, fall, and early winter months), still showed an appreciable percentage of viability clearly indicates that they can withstand adverse weather for some length of time. Results of a similar test have been reported by Yu (1933) who obtained conidia of *H. maydis* from diseased materials kept in the laboratory for three months and thereafter left on the surface of the soil for five months, a total period of eight months from the time the materials were collected. The materials had been exposed to the weather outside the laboratory. Of the total number of conidia tested, 0.5 and 8.1 per cent germinated in tap water alone and in tap water with bits of maize seedlings, respectively. Again, these results indicate the ability of the conidia of the fungus to survive for at least eight months the action of weather elements.

On the basis of these findings, it can be concluded that, in addition to the mycelium, the conidia produced on infected remains of maize in the field can also serve as primary inoculum for newly planted maize plants.

Methods of dissemination

Fungi, pathogenic or otherwise, are disseminated in different forms (spores, sclerotia, mycelia, etc.) by various agents such as air, rain, running water, insects, higher animals, and even man himself. Various investigators have studied particularly the aerial dissemination of microorganisms (Stakman *et al.*, 1922, 1923; Keitt, 1942).

A study of the aforementioned agents in relation to their ability to disseminate plant pathogens is important in interpreting the occurrence of a given plant disease in one locality and its absence in another. It is also important from the standpoint of plant disease prevention and control. In the present work the role of air, rain, and insects was determined in relation to the dispersal of conidia of *H. maydis*. The experiments were conducted during the summer of 1948 at the Atkins Garden, Soledad, Cuba.

Dissemination by air. Petri dishes with sterile water agar were exposed for 10 minutes in and at different distances away from a field planted to Cuban corn (maize Criollo) on which leaf spot was present. Two series of exposures were made, one in the morning and another in the afternoon, for two days when the air currents were just slightly perceptible. The exposed dishes were taken to the laboratory and examined microscopically for the presence of fungous spores. In all cases, conidia of *H. maydis* were among the spores caught. Spores of *Fusarium, Alternaria,* and what appeared to be a *Curvularia* were also observed. *H. maydis* spores were abundant in the dishes which were exposed at or near the center of the field and also in those exposed at the leeward edge of the field or a short distance (5 to 10 feet) away from it. The dishes exposed at some distance from the field (50 feet) still caught a good number of spores. The plates located to windward invariably caught few spores or none at all. Moreover, the few spores observed were present only in the plates exposed at or very near the windward edge of the field.

From the exposed plates, several isolations of *H. maydis* were made using the single-spore technique. All the isolates sporulated vigorously on potato-dextrose agar slants. Conidia from two-week-old cultures of these isolates were subsequently used for inoculating potted maize seedlings and proved to be pathogenic. All of them produced the typical spotting on the leaves. No attempt was made to isolate and test the pathogenicity of the other species of fungi caught in the dishes along with *H. maydis*.

Another experiment similar to the one just described was performed in a field planted to sweet corn variety U.S.D.A. 34. The results were essentially the same as those obtained in the preceding experiment. There were more plants infected with leaf spot in the sweet corn field; consequently, more *H. maydis* spores were caught here than in the field planted to Cuban corn.

In another experiment to prove that *H. maydis* spores can be disseminated by wind, glass slides thinly coated with vaseline were used. Petri dishes, each of which contained two slides, were placed at different points in and away from the maize field. The dish covers were removed so that the slides inside were exposed for 10 minutes to the prevailing wind. Microscopic examination revealed that of the 30 slides which were exposed, 7 did not show any *H. maydis* spores. The rest showed the presence of spores. The slides which did not show any spores were those located 20 to 30 feet to windward of the field. Those that caught the spores had been exposed at the leeward edge of the field or at a distance of 30 to 50 feet away from it. The slides situated at or very near the windward edge caught a few spores.

These results corroborate those obtained from spore-catching experiments conducted by the writer in 1944 in Los Baños. His experiments were conducted in fields planted to the native maize varieties, Calauan Yellow Flint and Lagkitan, both of which are susceptible to leaf spot. It was observed that conidia of *H. maydis* caught in exposed Petri dishes were particularly abundant two to three days after a heavy rain. This finding can be attributed to the fact that the increase in humidity following a heavy rain is very conducive to the sporulation of the fungus. The spores produced are dislodged easily and carried away even by slight currents of air.

From the results of the foregoing experiments, it is apparent that air plays a major role in the dispersal of spores of *H. maydis* within short distances and possibly for long distances when winds of high velocity occur at the time when spores are abundant. The spores thus dispersed can cause infections of maize plants in the same field or in a neighboring one and perhaps in fields which are distantly located.

Dissemination by rain. Dissemination of conidia by rain was studied. Droplets of rain water caught in sterile Petri dishes which had been exposed in the immediate vicinity of infected plants were found to contain numerous conidia of *H. maydis.* Evidently these droplets laden with conidia, when splashed from leaves or from the ground, can account, at least partly, for the infection of nearby plants. The spread of the disease resulting from the above method of spore dispersal is quite limited in comparison to that resulting from dissemination by wind, and usually only the plants immediately surrounding the source of inoculum are involved. The range of spore dispersal by rain droplets, however, undoubtedly becomes more extensive when the rain is accompanied by strong winds.

Dissemination by insects. During the writer's observation of maize fields at the Atkins Garden, he noticed the presence in extraordinary abundance of leaf hoppers (*Peregrinus maidis* Ashm.) in the early part of August, 1948. The leaf hoppers concentrated their feeding on the sweet corn plants, which were mostly infected with

H. maydis. The question arose, therefore, as to the possibility of the insects spreading the disease from one plant to another; hence, the following experiments were performed to determine whether the leaf hoppers are able to disseminate the conidia of *H. maydis*.

The hoppers were caught by means of a small insect net and placed in test tubes of sterile water (10 individuals to a tube containing 10 ml. of water) which were shaken vigorously to dislodge the spores which might be adhering to the appendages and bodies of the insects. The insects were then removed from the tubes and the resulting suspensions examined drop by drop under the microscope. Suspensions from a total of 15 test tubes were examined and they all yielded conidia of *H. maydis*, ranging from a minimum of 3 spores to a maximum of 17 spores to a tube.

In another experiment, the captured leaf hoppers were placed inside Petri dishes containing sterile potato-dextrose agar and allowed to crawl on the surface of the agar for 30 minutes. Afterwards the insects were removed and the dishes were incubated for 72 hours. On 11 out of 15 agar plates thus treated, colonies of *H. maydis* appeared together with colonies of other species of fungi, notably *Fusarium*. Pure cultures of *H. maydis* were prepared from the incubated plates. These were used later in inoculation experiments and were proved pathogenic on maize.

While these experiments were in progress, seeds of a number of maize strains received from Mexico were planted in experimental plots adjacent to the sweet corn field where the hoppers were being collected. At the end of three weeks, when the Mexican maize seedlings were about a foot high, the hoppers switched their depredation from the old corn field to the new plots. Symptoms of leaf spot started to appear on the corn seedlings. At the age of five weeks almost all the plants in the new plots showed the disease in varying degrees of severity. A count on five representative plots, each containing 20 hills, was made and the percentage of infection was found to range from approximately 30 to as high as 90 per cent.

From the new plots, leaf hoppers were again collected and examined for the presence of conidia of *H. maydis*. The results were all positive.

It is evident then that insects such as *Peregrinus maidis* and possibly others that feed on maize can be effective agents in the local spread of leaf spot of maize. Not only do they serve as mechanical transports for the spores of the pathogen, but they also weaken the host plants to some extent through their feeding, thereby predisposing them to infection by fungous parasites. These sucking insects also provide points of entry for plant pathogens through the numerous punctures that they inflict upon the tissues of the host plants.

By way of summarizing the methods of dissemination of conidia of *H. maydis*, it may be said that three natural agents—wind, rain,

and insects—have been proved to assume a role in the spread of the maize leafspot pathogen, wind apparently being the most important agent.

CONTROL MEASURES

Laboratory tests of protectant fungicides

In the present investigation, the effects of four protectant fungicides on *H. maydis* were tested. These fungicides, namely, commercial Bordeaux mixture, Spergon, Semesan, and Semesan Jr., have been highly advertised as effective against various diseases of maize, vegetables, and ornamental plants. The tests, therefore, were performed (1) to determine the lowest concentration of each fungicide that would be lethal to the conidia of *H. maydis* and (2) to test whether this concentration would adequately protect maize plants against the disease in the greenhouse.

The fungicidal tests were perfomed according to the slide-germination method recommended by the Committee on Standardization of Fungicidal Tests, American Phytopathological Society (1943). The fungicide to be tested, diluted to the desired strength, was sprayed as uniformly as possible on a 3 × 1 inch chemically clean glass slide. Since the slide had not been subjected to a "weathering" or "rain" test, coating it with a special surfacing substance like cellulose nitrate was not necessary. A uniform deposition of the fungicide on the slide was secured by four successive squirtings with a DeVilbiss No. 15 atomizer containing the solution of fungicide, the nozzle being held 6 inches directly above the slide. The sprayed slide was allowed to dry, usually for three hours under a small bell jar, and then placed upon a glass-rod support (glass rod bent into a U) inside a moist chamber. The writer found it satisfactory to use as moist chamber a large Petri dish with a diameter of 145 mm. and bottom-lined with two sheets of moist filter paper. Four drops of spore suspension, equidistant to each other, were applied to the slide with a 2-cc. sterile pipette, each drop being approximately 0.05 cc. in volume and spreading to a diameter of about 10 mm. The spores used in the tests were taken from two-week-old potato-dextrose agar slant cultures of the Pennsylvania isolate of the fungus and suspended in sterile distilled water. The density of the spore suspension was adjusted so that the 0.05-cc. drop of suspension contained approximately 50 spores. Four slides bearing the fungicide and drops of spore suspension were accommodated in each moist chamber and incubated at a temperature of 27°C. for 24 hours, after which time the slides were examined for germinating spores. The percentage of germination was calculated on the basis of 200 spores for each dilution of the different fungicides. Controls were maintained by pipetting four drops of spore suspension (same volume and density as specified previously) on chemically clean slides, incubating them at the same temperature for the same length of time.

The percentage of germination was compared with that of the treated slides.

The results of the slide-germination tests showed that Bordeaux mixture totally suppressed germination of conidia at a dilution as high as 1:1000, whereas the other three fungicides at the same dilution allowed considerable conidial germination. Semesan could be considered second to Bordeaux mixture in effectivity as it completely prevented conidial germination at a dilution of 1:500. At this dilution, Spergon and Semesan Jr. were still not entirely lethal to the conidia of the fungus, for they gave 6.5 and 9.0 per cent germination, respectively. Spergon seemed to be merely slightly more effective than Semesan Jr. as revealed by the small differences in percentage of germination obtained with these two fungicides at dilutions ranging from 1:500 to 1:1500. The four fungicides tested could be ranked, with respect to the highest dilution at which germination of conidia was completely suppressed, as first, Bordeaux mixture; second, Semesan; third, Spergon; and fourth, Semesan Jr.

Fungicidal tests on maize plants in the greenhouse

The real and ultimate test of the effectivity of any fungicide against a given plant disease is to apply it in the field. In the present study, however, spraying experiments in the field were not feasible; hence, the protective value of the fungicides tested was determined only on maize plants grown in the greenhouse.

Since laboratory tests had shown that Bordeaux mixture and Semesan were promising as protectant fungicides against *H. maydis,* the writer used these two in greenhouse experiments. One-month-old maize plants of the variety Golden Bantam, growing in pots in the greenhouse, were sprayed by means of a hand spray gun with either a 1:1000 solution of Bordeaux mixture or a 1:500 solution of Semesan. These dilutions were tried since they had been found to be the highest dilutions of Bordeaux mixture and Semesan, respectively, which completely suppressed germination of conidia in the slide-germination tests. One day after the plants had been sprayed with the fungicides, they were artificially inoculated by means of a DeVilbiss atomizer with a heavy spore suspension of the Pennsylvania isolate. The inoculum was taken from two-week-old potato-dextrose agar slant cultures and suspended in sterile distilled water. Controls consisted of unsprayed maize plants also inoculated with the fungus. All plants were placed under large bell jars for the first 36 hours after inoculation and then left uncovered on a bench in the greenhouse and observed for the development of the disease. Final observations were made two weeks after inoculation.

In the preliminary experiments some of the plants sprayed with either 1:1000 solution of Bordeaux mixture or 1:500 solution of Semesan developed infections, although to a much lesser extent than those developed on the unsprayed inoculated plants. This indicates that the concentration of the fungicide which proved lethal to the fungus

on glass slides was not equally effective against the fungus on the host tissues, a fact which seems to confirm Marsh's ((1936) observation that fungicides were less effective when tested on leaves. Part of this disparity may perhaps be ascribed to the stimulating effect of leaf excretions on fungous spore germination as previously observed by Wilcoxon and McCallan (1934). In view of the preceding observations, the writer tested the two fungicides at lesser dilutions. In all the later tests, the procedure followed was the same as in the earlier ones.

The results of fungicidal tests on maize plants in the greenhouse showed that spraying with Bordeaux mixture diluted to 1:1000 and 1:800 and with Semesan diluted to 1.500 and 1:300 did not protect the plants completely against infection by *H. maydis*. Application of a 1:500 dilution of Bordeaux mixture or a 1:200 dilution of Semesan, however, absolutely protected the plants against the disease. At these dilutions neither of the two fungicides exerted any deleterious effect on the host. Whether the same dilutions would be equally effective against the disease in the field remains to be investigated. Despite the limited results obtained by the writer from his tests in the greenhouse, Bordeaux mixture seems to be more suitable for use since its protectant property is markedly greater than that of Semesan. This is evidenced by the fact that at a dilution more than twice that of Semesan, Bordeaux mixture afforded complete protection to the host plants.

Sanitation

The fungus has been found to be capable of living as a saprophyte on infected remains of maize in the field for several months at least. It would be advisable, therefore, to collect and burn the heavily infected remains of plants after the season's harvest; otherwise they would serve as source of primary inoculum for succeeding maize crops.

The results of cross-inoculation work indicate that members of Gramineae other than maize can be infected by the pathogen and, hence, can serve as potential reservoirs of infection for maize. All probable wild hosts, therefore, growing in maize fields and vicinity should be destroyed as far as practicable.

Planting of resistant varieties of maize

Although the numerous varieties of maize observed by the writer were infected by the fungus, the disease was not equally severe on all of them. For instance, in 1943 and 1944 in the Philippines, the variety Lagkitan was observed to be more severely infected than other native varieties growing in the same field during the same season. In the summer of 1948 in Cuba, the writer noted that several Mexican strains of maize were remarkably susceptible to leaf spot, whereas Cuban corn (maize Criollo) was only slightly affected. Wernham (1946) reported that in Pennsylvania inocula-

tion tests proved that the inbred lines of maize (Ind 461-3 and Ind Tr) which were highly susceptible to *H. turcicum* were markedly resistant to *H. maydis*. From the foregoing accounts, it seems possible that resistant varieties of maize might be developed. Planting such resistant varities would be the most practical and effective control against the disease.

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TABLE 1

Variations in size and septation of conidiophores of the Philippine isolate of Helminthosporium maydis

SOURCE OF CONIDIOPHORES	NUMBER OF CONIDIOPHORES MEASURED	LENGTH			WIDTH			NO. OF SEPTA		
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
		μ	μ	μ	μ	μ	μ			
Naturally infected leaves not incubated in a damp chamber	50	98.6	241.7	198.6	4.5	7.9	5.9	3	11	5.8
Naturally infected leaves incubated in a damp chamber	100	102.5	376.5	271.3	4.7	8.5	6.4	3	18	7.6
Corn meal agar plate cultures	100	106.2	549.4	307.5	4.6	8.1	6.1	4	22	12.9

TABLE 2

Variations in size and septation of conidia of the Philippine isolate of Helminthosporium maydis

SOURCE OF CONIDIA	NUMBER OF CONIDIA MEASURED	LENGTH			WIDTH			NO. OF SEPTA		
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
		μ	μ	μ	μ	μ	μ			
Naturally infected leaves not incubated in a damp chamber	100	42.5	127.4	86.9	9.3	15.9	13.8	4	11	7.8
Naturally infected leaves incubated in a damp chamber	100	43.8	133.5	87.2	9.8	16.5	14.2	5	11	8.3
Corn meal agar plate cultures	100	55.3	135.2	89.7	10.2	17.3	14.9	5	12	8.6

TABLE 3

Methods of germination of conidia of Helminthosporium maydis

METHOD OF GERMINATION	PERCENTAGE OF GERMINATION
1 germ tube from each end	56.2
1 germ tube from one end	33.6
2 germ tubes from one end	3.8
1 germ tube from one end and 2 from the other end	3.2
2 germ tubes from each end	1.8
1 germ tube from one end and 1 from an intermediate segment	0.8
1 germ tube from each end and 1 from an intermediate segment	0.4
2 germ tubes from one end and 1 from an intermediate segment	0.2
Total	100.0

TABLE 4

Cellulolytic activity of Helminthosporium maydis grown on strips of muslin cloth supplied with nutrients in "Formula A"

ISOLATE	GROWTH AT END OF				AVERAGE TENSILE STRENGTH IN gm. AT END OF 4 WEEKS
	1 week	2 weeks	3 weeks	4 weeks	
Philippine	+ [a]	++ [b]	+++ [c]	++++ [d]	261.0
Pennsylvania	+	+++	+++	++++	254.5
Beltsville	+	++	+++	+++	273.0
Control	−	−	−	−	Able to withstand 2000 gm. without breaking.

[a] Slight fuzziness clearly visible only with a hand lens.
[b] Increased fuzziness visible to the naked eye.
[c] More growth with sparse sporulation.
[d] Well-developed growth with increased sporulation.

TABLE 5

Effect of carbon source on the growth of Helminthosporium maydis in Czapek's sodium nitrate solution

CARBON SOURCE	MYCELIAL GROWTH OF THREE ISOLATES AFTER 4 WEEKS		
	Philippine	Pennsylvania	Beltsville
No carbon	+ [a]	+	+
Filter paper	++ [b]	+++	++
Cellobiose	+++ [c]	++++	+++
Dextrose	++++ [d]	++++	++++

[a] Scarce.
[b] Fairly abundant.
[c] Abundant.
[d] Very abundant.

TABLE 6

Effect of temperature on mycelial growth and sporulation of Helminthosporium maydis

TEMP. °C.	LINEAR GROWTH IN mm. AFTER:[a]				AVERAGE DAILY GROWTH IN mm.	RELATIVE ABUNDANCE OF AERIAL MYCELIUM AND CONIDIA AFTER 8 DAYS	
	2 days	4 days	6 days	8 days		Aerial mycelium	Conidia
5	— [b]	—	—	—	—	—	—
10	6.8	14.4	22.6	27.2	3.40	+ [d]	—
15	16.0	35.1	51.9	61.3	7.66	++ [e]	+
20	25.7	52.3	80.1	97.7	12.21	++	++
24	41.6	81.9	119.5	158.3	19.79	+++ [f]	+++
27	55.6	104.4	152.8	192.5	24.06	+++	++++
30	64.8	124.5	180.0	221.3	27.66	++++ [g]	++
33	41.4	86.1	126.2	146.7	18.34	+++	+
35	15.4	31.8	47.8	58.2	7.28	++	—
37	3.7	6.2	11.5	15.9	1.99	+	—
40	—	—	—	2.4	0.30	± [c]	—

[a] Each linear growth figure is the average of 2 series of 4 cultures each.
[b] No growth and/or no conidia.
[c] Hardly noticeable.
[d] Very scarce.
[e] Scarce.
[f] Abundant.
[g] Very abundant.

TABLE 7

Effect of temperature on size and septation of conidia of Helminthosporium maydis

TEMP. °C.	NO. OF CONIDIA MEASURED	LENGTH			WIDTH			NO. OF SEPTA		
		Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
		μ	μ	μ	μ	μ	μ			
15	100	23.7	85.2	53.5	7.3	14.5	10.4	3	8	5.4
20	100	30.4	103.6	78.3	9.6	16.7	13.2	3	10	7.9
24	100	35.7	101.8	85.2	10.2	16.8	13.8	4	11	8.1
27	100	38.2	112.7	83.6	9.7	17.0	14.5	5	12	8.8
30	100	31.3	104.1	68.7	7.1	16.5	11.7	3	10	6.3
33	100	18.5	83.7	49.8	5.4	15.9	8.3	1	7	4.5

TABLE 8

Effect of temperature on the germination of conidia of Helminthosporium maydis

TEMPERATURE IN °C.	PERCENTAGE OF GERMINATION
5	0.0
10	4.0
15	55.5
20	78.0
24	88.5
27	94.0
30	96.5
33	91.5
35	84.5
37	5.0
40	0.0

TABLE 9

Effect of pH on mycelial growth and sporulation of Helminthosporium maydis

pH	DIAMETER [a] OF COLONY IN mm. AFTER				AVERAGE DAILY GROWTH IN mm.	RELATIVE ABUNDANCE OF AERIAL MYCELIUM AND CONIDIA AFTER 8 DAYS	
	2 days	4 days	6 days	8 days		Aerial mycelium	Conidia
1.2	— [b]	—	—	—	—	—	—
1.6	—	—	—	—	—	—	—
2.2	4.7	16.2	32.7	37.5	4.69	+ [c]	—
2.8	6.5	20.8	39.1	46.0	5.75	+	—
3.4	7.8	25.7	45.6	53.1	6.64	+ + [d]	+
4.0	8.5	26.6	50.2	68.2	8.53	+ + + [e]	+
4.6	10.0	28.9	53.3	74.0	9.25	+ + + + [f]	+ +
5.2	13.7	30.8	55.9	77.1	9.64	+ + + +	+ + +
5.8	17.0	34.1	57.0	79.0	9.88	+ + + +	+ + + +
6.4	15.0	30.2	49.1	73.2	9.15	+ + + +	+ + + +
7.0	14.5	27.3	48.0	65.0	8.13	+ + +	+ + +
7.6	13.6	25.9	44.6	54.5	6.81	+ + +	+ + +
8.2	11.7	26.4	46.3	59.4	7.43	+ + +	+ + +
8.8	9.0	23.2	42.3	52.6	6.58	+ +	+ +
9.4	7.5	19.1	34.7	41.7	5.21	+ +	+ +
10.0	5.3	14.9	27.6	36.4	4.55	+	+
10.6	4.3	12.0	22.1	31.6	3.95	+	+
11.2	—	8.3	18.0	25.4	3.18	+	—
11.8	—	—	—	—	—	—	—

[a] Each diameter reading is the average of 2 series of 3 cultures each.
[b] No growth and/or no conidia.
[c] Very scarce.
[d] Scarce.
[e] Abundant.
[f] Very abundant.

TABLE 10

Ratings of infection of the four isolates of Helminthosporium maydis

AGE OF PLANTS INOCULATED	VARIETY GOLDEN BANTAM				VARIETY SWEEPSTAKES			
	Penn.	Phil.	Belts.	Cuban	Penn.	Phil.	Belts.	Cuban
10 days	100.00	100.00	100.00	83.33	100.00	100.00	97.77	86.11
20 days	100.00	100.00	100.00	86.66	90.00	88.88	83.33	72.73
30 days	94.44	86.66	66.66	46.66	93.33	85.71	57.14	48.14
40 days	71.42	66.66	63.88	53.33	86.11	70.37	64.44	48.88
Average rating of infection	91.46	88.33	82.63	67.49	92.36	86.24	75.67	63.96

TABLE 11

Results of inoculating other aerial parts of maize plants with Helminthosporium maydis

NO. OF PARTS INOCULATED	PARTS INOCULATED	TREATMENT	RESULTS
5	Stems	Uninjured; not kept moist	No infection
5	Stems	Uninjured; kept moist	No infection
5	Stems	Injured; not kept moist	No infection
5	Stems	Injured; kept moist	4 infected; 1 with incipient lesion
7	Midribs	Uninjured; not kept moist	No infection
6	Midribs	Uninjured; kept moist	No infection
8	Midribs	Injured; not kept moist	No infection
10	Midribs	Injured; kept moist	All infected
8	Leaf sheaths	Uninjured; not kept moist	All infected
10	Leaf sheaths	Uninjured; kept moist	All infected
5	Ears with bracts intact	Uninjured; not kept moist	All infected
5	Ears with bracts intact	Uninjured; kept moist	All infected
5	Tassels	Not kept moist	No infection
5	Tassels	Kept moist	No infection

TABLE 12

Results of planting maize seeds in soil inoculated with Helminthosporium maydis

ISOLATE USED AS INOCULUM	NUMBER OF SEEDS PLANTED	NUMBER OF SEEDS GERMINATED	PERCENTAGE OF GERMINATION	NUMBER OF PLANTS ALIVE AFTER 1 MONTH	PRESENCE OF ROOT LESIONS
Pennsylvania	50	32	64	27	+
Philippine	50	37	74	33	+
Beltsville	50	36	72	35	+
Cuban	50	41	82	39	+
Control (soil + medium)	50	49	98	49	—
Control (soil alone)	50	50	100	50	—

TABLE 13

Results of inoculating different species of Gramineae with Helminthosporium maydis

PLANT INOCULATED	RESULTS [a]
Andropogon aciculatus Retz.	—
A. halepensis (L.) Brot. var. *propinquus* (Kunth) Merr.	—
A. sorghum (L.) Brot.	—
Avena sativa L.	—
Cymbopogon nardus (L.) Rendle	—
Cynodon dactylon (L.) Pers.	—
Digitaria corymbosa Roxb.	—
D. sanguinalis (L.) Scop. var. *australis* Spreng.	—
Eleusine indica (L.) Gaertn.	—
Eragrostis amabilis (L.) Wight and Arn.	—
Euchlaena mexicana Schrad.	++
Imperata cylindrica (L.) Beauv.	—
Leersia hexandra Swartz.	—
Oryza sativa L.	—
Panicum distachyum L.	—
P. flavidum Retz.	—
P. maximum Jacq.	—
Paspalum conjugatum Berg.	—
Rottboellia exaltata L.f.	+
Saccharum officinarum L.	±
S. spontaneum L.	—
Setaria italica (L.) Beauv.	—
Sorghum vulgare var. *sudanense* (Piper) Hitchc.	—
Sporobolus indicus (L.) R. Br.	—
Tricholaena rosea Nees.	—
Tripsacum latifolium Hitchc.	±
T. laxum Nash	±
Triticum aestivum L.	—

[a] ++ Susceptible.
+ Less susceptible.
± Very slightly susceptible.
— No infection.

TABLE 14

Variation in length of germ tubes at time of penetration and frequency of occurrence of stomatal and direct epidermal penetrations

LENGTH OF GERM TUBES (in microns)	NO. OF GERM TUBES OBSERVED	NO. OF GERM TUBES PENETRATING THROUGH	
		Stomata	Epidermis
20.5– 30.5	2		2
35.5– 45.5	7	1	6
51.5– 61.5	5	3	2
72.5– 82.5	4	2	2
93.5–103.5	9	3	6
105.5–115.5	8	5	3
125.5–135.5	7	5	2
145.5–155.5	6	1	5
180.5–190.5	9	5	4
200.5–210.5	7	4	3
225.5–235.5	3		3
285.5–295.5	4	1	3
300.5–310.5	6	2	4
330.5–340.5	3	1	2
Total	80	33	47

DESCRIPTION OF PLATES

PLATE I

1.—Portions of mature maize leaves showing lesions caused by *Helminthosporium maydis*. Note the typical spotting effect and the coalescence of lesions along the margins of the leaves resulting in the production of extensive necrotic areas. Note also the dark-gray specks on the surface of the lesions. These specks consist of conidiophores and conidia of the fungus. × ½.

2.—Inoculated and control 25-day-old seedlings of sweet corn variety Golden Bantam. Inoculation was done by spraying the plants with conidial suspension of the Pennslyvania isolate of *H. maydis;* the control was sprayed with sterile distilled water. Both inoculated and control plants were placed in a damp chamber for the first 36 hours, then transferred to a bench in the greenhouse and photographed 4 days later. × 1/6.

3.—Two conidiophores which have emerged singly from the stomata and a tuft of 3 which has emerged in the same manner. × 216.

4.—Same as (3), but photographed to show completely the group of 3 conidiophores. × 216.

5.—A tuft of 3 conidiophores of the Philippine isolate of *H. maydis*. Prepared from naturally infected maize leaf incubated in a damp chamber. ×120.

6.—Conidiophores of the Pennsylvania isolate of *H. maydis* which have emerged from the stomata singly and in tufts of 2 and 3. Prepared from naturally infected maize leaf incubated in a damp chamber. × 105.

PLATE II

1.—A tuft of 2 conidiophores and a single conidiophore above. × 216.

2.—A tuft of 4 conidiophores which has emerged from a stoma. × 216.

3.—A young conidiophore arising from a hyphal filament and bearing a young terminal conidium. × 195.

4.—An older conidiophore arising from a hyphal filament and bearing a mature terminal conidium. × 195.

5.—One conidium germinating by the production of a germ tube at one end. The other shows 2 germ tubes at one end—an abnormal method of germination. × 233.

6.—Conidiophores and conidia of the Philippine isolate of *H. maydis* produced in a 10-day-old potato-dextrose agar slide culture. × 87. Note the short branch at the left bearing 3 conidia, 2 of which are still attached and 1 is already dislodged. × 87.

7.—Conidiophores and conidia of the Pennsylvania isolate of *H. maydis* produced in a 10-day-old potato-dextrose agar slide culture. × 87.

PLATE III

1.—Mature conidiophore with mature conidia attached to the prominent geniculations which occur at rather wide intervals. Note the conidium initial starting to develop at the tip of the conidiophore. × 195.

2.—Same description as (1), but note the 2 very young conidia being formed at the tip of the conidiophore at the right. × 195.

3.—Conidial germination by the production of 2 germ tubes at one end and 1 tube at the other end. ×233.

4.—Two conidia showing normal method of germination. The conidium below shows atypical germination by the production of 2 germ tubes at one end and 1 tube at the other end. × 233.

5.—Germination by the production of 2 germ tubes at each end of the conidium. × 233.

6.—Germination by the production of 1 germ tube at one end and another germ tube from an intermediate segment. × 233.

7.—Conidium showing typical bipolar germination. × 233.

8.—Germination by the production of 1 germ tube at each end and another tube from an intermediate segment. × 233.

9.—Conidial germination, typical and atypical. The conidium below shows atypical germination; the other three show typical method of germination. × 233.

10.—Section of artificially inoculated maize leaf showing direct epidermal penetration by a germ tube. × 128.

11.—Same as (10), but much enlarged to show the formation of an ovoid appressorium at the tip of the germ tube. × 640.

ORILLO: HELMINTHOSPORIUM DISEASE OF MAIZE

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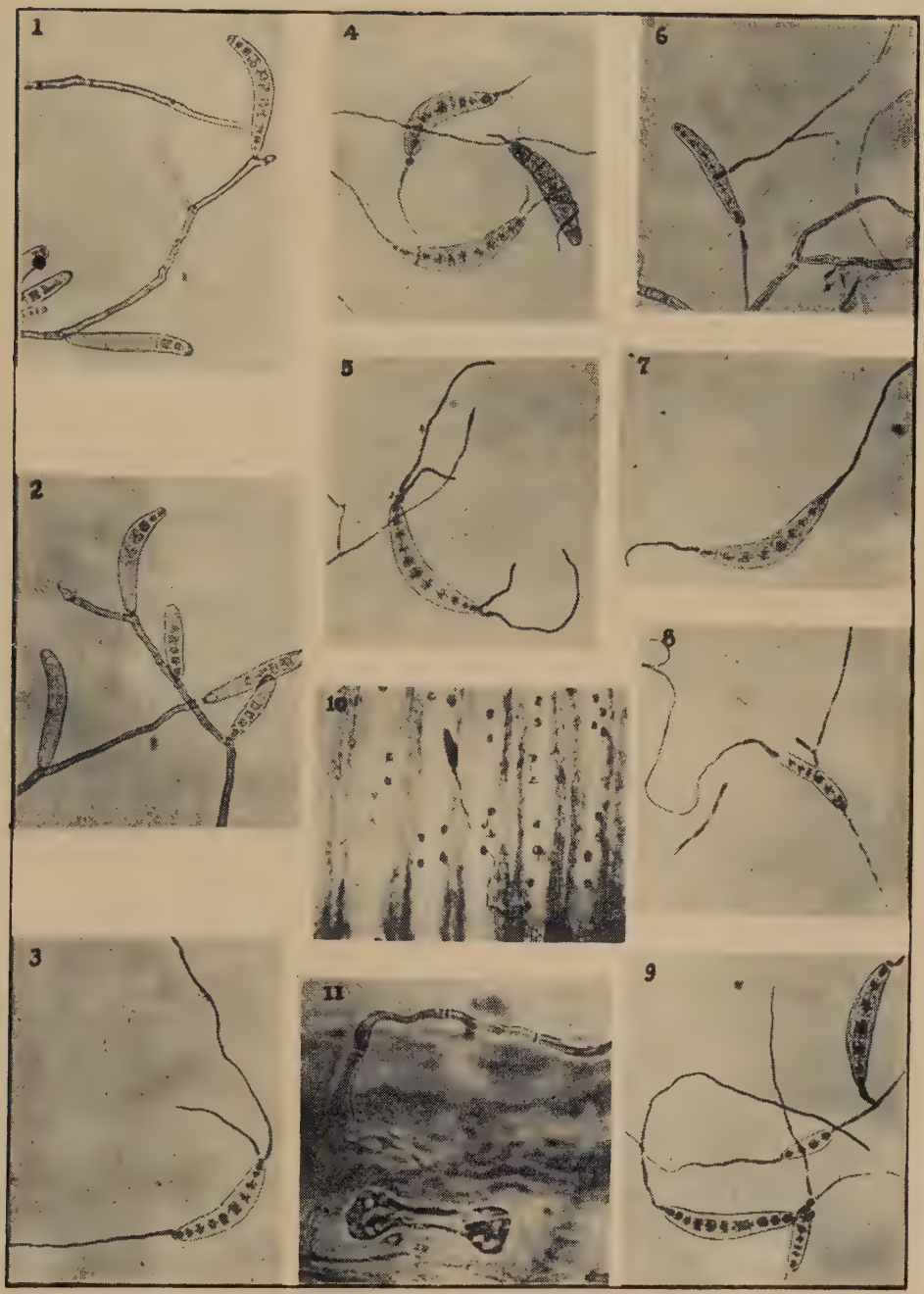

ORILLO: HELMINTHOSPORIUM DISEASE OF MAIZE

COLLEGE AND ALUMNI NOTES

Dr. Frederick L. Wellman of the Office of Foreign Agricultural Relations, USDA, detailed to the Inter-American Institute of Agricultural Sciences in Turrialba, Costa Rica, and Dr. William H. Cowgill of the Instituto Agropecuario Nacional in Guatemala visited the College on November 13, 1952, to arrange for cooperative planting in this College to breed rust-resistant Arabian coffee. Mr. John V. Hepler accompanied the guests.

Another recent campus visitor was Miss Margaret Flory, secretary for student work of the Board of Foreign Mission of the Presbyterian Church, New York, U.S.A. Miss Flory is looking into prospects of sending American students to this College for part of their collegiate work. She was accompanied by Rev. Paul R. Dotson, new pastor of the College Protestant Church, and Miss Jeannie M. Dodge of Bridgeport, Connecticut.

Mr. P. A. Duclayan of the National Power Corporation, detailed to the PHILCUSA, visited the campus on November 18 to look into the feasibility of installing an electric generating plant to serve both the Forestry and Agriculture campuses. Mr. Duclayan was accompanied by Dean Leland E. Call and Director Florencio Tamesis.

Dr. C. A. Bratton, Prof. N. B. Tablante, and Dr. F. M. Sacay are cooperating with the Agricultural Credit and Cooperative Financing Administration in preparing lectures for an institute to be held in Manila this month. The institute will be open to applicants for positions in the ACCFA.

Agricultural Education 105, a course in methods of extension teaching, is being offered this semester with Professor Montgomery Robinson in charge. Members of the Cornell group and personnel of the MSA and of the Bureau of Agricultural Extension will also give lectures. At present, 32 students are taking the course.

Students from Maryknoll Academy, Lucena, Quezon, visited the College on November 15, 1952. Miss Filomena Fortich and Mr. Juan Pancho, of the Department of Agricultural Botany, gave lecture-demonstrations on different phases of botanical studies and investigations. The students were accompanied by their principal, Sister Robert Marie.

In the meeting held on November 4, 1952, the faculty approved to recommend to the University Council the graduation of the following candidates for the degree of Bachelor of Science in Agriculture: Rafael R. Araneta, Jr., Buenaventura B. Bombay, Felipe V. Cagampang, Jose B. Cajigal, Agaton T. Carag, Agustin T. Castro, Alejandro R. Cuadro, Juanito P. Dimaano, Pablo A. Feliciano, Jr., Lilia V. Hosillos, Jose S. Mendoza, Domingo G. Molina, Pacifico P. Namocale, Brigido A. Narit, Prospero B. Pajarillaga, Jesus P. Ricahuerta, Vic-

tor C. Rimando, Reynaldo C. Rodriguez, Juanito S. Sarmenta, Antonio V. Valida, Gloria R. Vega, Romualdo V. Vijandre, Jr., Sotero V. Villanueva, Quirino N. Villaviza, and Rodolfo C. Ylarde.

Two students, Jose S. Mendoza and Jesus R. Ricahuerta, satisfactorily completed the requirements for the Certificate in Agricultural Education.

Mr. Paulino F. Cayco, '49, and Miss Estela Villegas, BSHE '51 (PWU), were united in marriage on October 5, 1952, at the Roman Catholic Church of Los Baños, Laguna. After the church ceremonies, breakfast was served at the Molawin Mess Hall. Calla flowers to decorate the church were presented by the following alumni of this College who are teaching in the Mountain National Agricultural School, La Trinidad, Mt. Province: Luis J. Dañgilan, '24, Francisco E. Mercado, '50, Doroteo C. Asuncion, '48, Florencio S. Caguioa, '50, Crispin E. Cabanilla, '48, Raymundo S. Laher, '38, Fernandico M. Cuaresma, 49, Mauro L. Ruma, '51, Juan S. Padilla, '32, Dolan T. Albas, '51, and Constancio T. Medrana, '27.

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